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(54) Title: SYNTHESIS OF CLASTO-LACTACYSTIN β-LACTONE AND ANALOGS THEREOF

(57) Abstract

The present invention is directed to an improved synthesis of *clasto*-lactacystin-β-lactone, and analogs thereof, that proceeds in fewer steps and in much greater overall yield than syntheses described in the prior art. The synthetic pathway relies upon a novel stereospecific synthesis of an oxazoline intermediate and a unique stereoselective addition of a formyl amide to the oxazoline. Also described are novel clasto-lactacystin-β-lactones, and analogs thereof and their use as proteosome inhibitors.

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Synthesis of Clasto-Lactacystin β-Lactone and Analogs Thereof

Background of the Invention

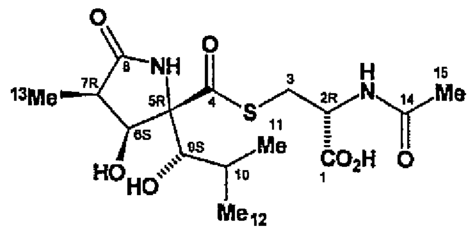
Field of the Invention

The invention relates generally to methods for preparing lactacystin and related compounds, to novel analogs of lactacystin and *clasto*-lactacystin β-lactone, and their uses as proteasome inhibitors.

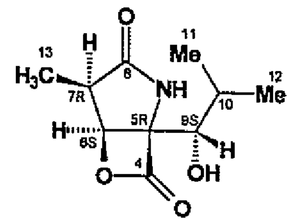
Description of Related Art

The *Streptomyces* metabolite lactacystin (**1**) inhibits cell cycle progression and induces neurite outgrowth in cultured neuroblastoma cells (Omura *et al.*, *J. Antibiotics 44*:117 (1991); Omura *et al.*, *J. Antibiotics 44*:113 (1991); Fenteany *et al.*, *Proc. Natl. Acad. Sci. (USA) 91*:3358 (1994)). The cellular target mediating these effects is the 20S proteasome, the proteolytic core of the 26S proteasome, which is the central component of the ubiquitin-proteasome pathway for intracellular protein degradation. Mechanistic studies have established that lactacystin inhibits the proteasome through the intermediacy of the active species, *clasto*-lactacystin β-lactone (**2**), which specifically acylates the *N*-terminal threonine residue of the proteasome X/MB1 subunit (Fenteany, *et al.*, *Science*

268:726 (1995); Dick *et al.*, *J. Biol. Chem.* *271*:7273 (1996)). Lactacystin analogs are disclosed by Fenteany *et al.* (WO 96/32105).



1



2

The ubiquitin-proteasome pathway is involved in a variety of important physiological processes (Goldberg *et al.*, *Chemistry & Biology* *2*:503 (1995); Ciechanover *Cell* *79*:13 (1994); Deshaies, *Trends Cell Biol.5*:431 (1995)). In fact, the bulk of cellular proteins are hydrolyzed by this pathway. Protein substrates are first marked for degradation by covalent conjugation to multiple molecules of a small protein, ubiquitin. The resultant polyubiquitinated protein is then recognized and degraded by the 26S proteasome. Long recognized for its role in degradation of damaged or mutated intracellular proteins, this pathway is now also known to be responsible for selective degradation of various regulatory proteins. For example, orderly cell cycle progression requires the programmed ubiquitination and degradation of cyclins. The ubiquitin-proteasome pathway also mediates degradation of a number of other cell cycle regulatory proteins and tumor suppressor proteins (e.g., p21, p27, p53). Activation of the transcription factor NF-κB, which plays a central role in the regulation of genes involved in the immune and inflammatory responses, is dependent upon ubiquitination and degradation of an inhibitory protein, IκB-α (Palombella *et al.*, WO 95/25533). In addition, the continual turnover of cellular proteins by the ubiquitin-proteasome pathway is essential to the processing of antigenic peptides for presentation on MHC class I molecules (Goldberg and Rock, WO 94/17816).

The interesting biological activities of lactacystin and *clasto*-lactacystin β-lactone and the scarcity of the natural materials, as well as the challenging

chemical structures of the molecules, have stimulated synthetic efforts directed toward lactacystin and related analogs. Corey and Reichard *J. Am. Chem. Soc. 114*:10677 (1992); *Tetrahedron Lett. 34*:6977 (1993)) achieved the first total synthesis of lactacystin, which proceeded in 15 steps and 10% overall yield. The key feature of the synthesis is a stereoselective aldol reaction of a *cis*-oxazolidine aldehyde derived from *N*-benzylserine to construct the C(6)-C(7) bond. In the synthesis reported by (Uno *et al., J. Am. Chem. Soc. 116*:2139 (1994)), stereoselective Mukaiyama-aldol reaction of a bicyclic oxazolidine silyl enol ether intermediate derived from D-pyroglutamic acid is employed in C(5)-C(9) bond construction. This synthesis proceeds in 19 steps and 5% overall yield. Aldol reactions under basic conditions of a similar bicyclic oxazolidine intermediate form the basis of model studies reported by (Dikshit *et al., Tetrahedron Lett. 36*:6131 (1995)).

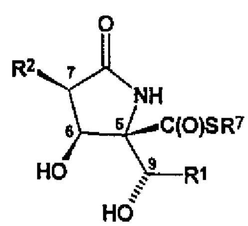
Aldol reactions of oxazoline-derived enolates feature prominently in the synthesis of lactacystin reported by Smith and coworkers (Suazuka *et al., J. Am. Chem. Soc. 115*:5302 (1993); Nagamitsu *et al., J. Am. Chem. Soc. 118*:3584 (1996)) and in the synthesis of (*6R*)-lactacystin reported by (Corey and Choi *Tetrahedron Lett. 34*:6969 (1993)); Choi Ph.D., Thesis, Harvard University, 44 (1995). In the former synthesis, which proceeds in 20 steps and 9% overall yield, the enolate is condensed with formaldehyde to install a single carbon atom, which must then be elaborated in a number of additional steps. In the Corey and Choi synthesis, the aldol reaction selectively provides the product of undesired stereochemistry, resulting in the eventual preparation of the C(6) epimer of lactacystin, which is devoid of biological activity.

Lactacystin has also been prepared in 22 steps and 2% overall yield from D-glucose (Chida *et al., J. Chem. Soc., Chem. Commun.* 793 (1995)). The biosynthetic pathway involved in production of the natural product has been investigated in feeding experiments involving ^{13}C-enriched compounds (Nakagawa *et al., Tetrahedron Lett. 35*:5009 (1994)).

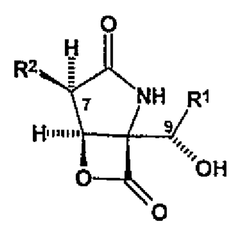
The reported syntheses of lactacystin are lengthy and proceed in low yield. Furthermore, none of these syntheses is readily adapted for analog synthesis. Thus, there is a need for improved methods for preparing lactacystin, *clasto*-lactacystin β-lactone, and analogs thereof.

Summary of the Invention

A first aspect of the present invention relates to a process for forming lactacystin or analogs thereof having Formula ***VI*** or *clasto*-lactacystin β-lactone or analogs thereof having Formula ***VII***:



VI



VII

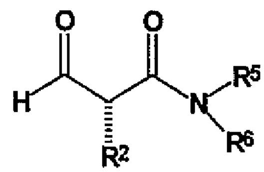
wherein

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^2 is alkyl, cycloalkyl, aryl, alkaryl, aralkyl, alkoxy, hydroxy, alkoxyalkyl, or amido, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted; and

R^7 is alkyl, aryl, aralkyl, alkaryl, wherein any of said alkyl, aryl, aralkyl or alkaryl can be optionally substituted.

A second aspect of the present invention is directed to a method of forming formyl amides of Formula ***XIV***:

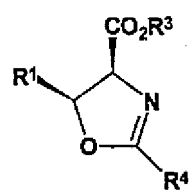


XIV

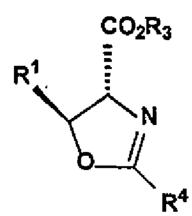
where R^2 is alkyl, cycloalkyl, aryl, alkaryl, aralkyl, alkoxy, hydroxy, alkoxyalkyl, or amido, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted; and

R^5 and R^6 are independently one of alkyl or alkaryl; or R^5 and R^6 when taken together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocyclic ring, which may be optionally substituted, and which optionally may include an additional oxygen or nitrogen atom.

A third aspect of the present invention relates to forming tri-substituted oxazolines of Formula ***Ia*** or ***Ib***:



Ia



Ib

where R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted; and R^4

is aryl or heteroaryl, either of which may be optionally substituted. The tri-substituted oxazolines of Formulae ***Ia*** and ***Ib*** are useful as starting materials in forming lactacysin, *clasto*-lactacystin β-lactone or analogs thereof via the process described herein.

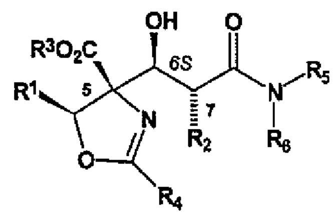
A fourth aspect of the present invention is directed to lactacysin, *clasto*-lactacystin β-lactone or analogs of Formulae ***VI*** and ***VII*** that possess unexpected biological activity. Lactacystin, *clasto*-lactacystin β-lactone, and analogs thereof possess biological activity as inhibitors of the proteasome. They can be used to treat conditions mediated directly by the function of the proteasome, such as muscle wasting, or mediated indirectly via proteins which are processed by the proteasome, such as the transcription factor NF-κB.

A fifth aspect of the present invention relates to pharmaceutical compositions, comprising a compound of Formula ***VI*** or Formula ***VII***, and a pharmaceutically acceptable carrier or diluent.

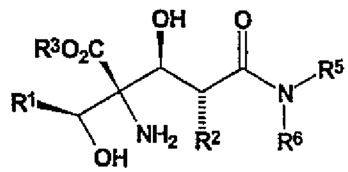
A sixth aspect of the present invention relates to methods of inhibiting proteasome function or treating a condition that is mediated directly or indirectly by the function of the proteasome, by administering a compound of Formula ***VI*** or Formula ***VII*** that possesses unexpectedly high activity in inhibiting the proteasome. Preferred Embodiments are directed to the use of a compound of Formulae ***VI*** or ***VII*** to prevent or reduce the size of infarct after vascular occlusion for example, for treating neuronal loss following stroke. An additional preferred embodiment is directed to the use of said compounds for treating asthma.

A seventh aspect of the invention relates to enantiomerically-enriched compositions of formyl amides of Formula ***XIV***.

An eighth aspect of the present invention relates to novel individual intermediates, such as aldols of Formula ***II*** and aminodiols of Formula ***III***:



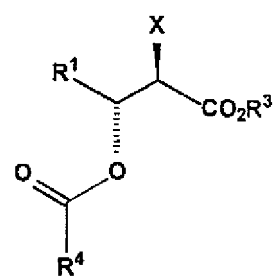
II;



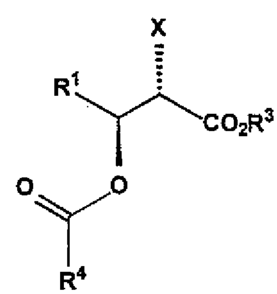
III

and individual steps within the multistep process for forming lactacystin, clasto-lactacystin β-lactone or various analogs thereof.

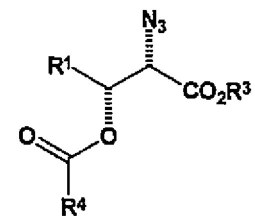
A ninth aspect of the present invention relates to individual intermediates, such as compounds of Formulae *XVII*, *XVIII* and *XIX*:



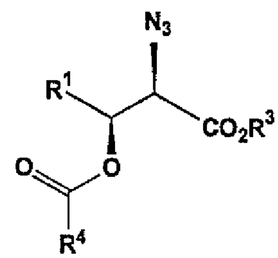
XVIIa



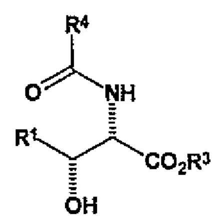
XVIIb



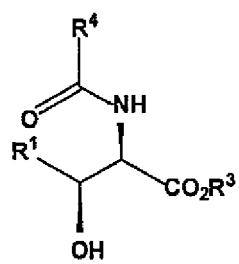
XVIIIa



XVIIIb



XIXa



XIXb

where X is a halogen, preferably Cl, Br or I, as well as individual steps within the multistep process for forming substituted oxazolines of Formula *I*.

Other features or advantages of the present invention will be apparent from the following detailed description, and also from the appending claims.

Brief Description of the Drawings

FIG. 1. depicts a graph showing the effect of compound **3b**, administered i.v., on infarct volume in rats (n=6-8).

FIG. 2. depicts a graph showing the effect of compound **3b**, administered i.v. on neurological score in rats (n=6-8).

Detailed Description of the Preferred Embodiments

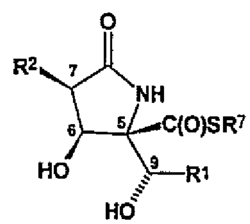
The present invention relates to an improved multi-step synthesis of lactacystin, *clasto*-lactacystin β-lactone, and analogs thereof, that proceeds in fewer steps and in much greater overall yield than syntheses described in the prior art. A number of individual process steps and chemical intermediates distinguish this synthetic pathway from pathways described in the prior art. For example, this synthetic pathway relies upon a novel stereospecific synthesis of an oxazoline intermediate, and a unique stereoselective addition of a formyl amide to the oxazoline.

The invention is also directed to novel analogs of Formulae *VI* and *VII* that possess unexpected biological activity. Lactacystin, *clasto*-lactacystin β-lactone, and analogs thereof possess biological activity as inhibitors of the proteasome. They can be used to treat conditions mediated directly by the function of the proteasome, such as muscle wasting, or mediated indirectly via proteins which are processed by the proteasome, such as the transcription factor NF-κB. The present invention is also directed to methods of inhibiting proteasome function or treating a condition that is mediated directly or indirectly by the function of the proteasome, by administering a compound of Formula *VI* or *VII* that possesses unexpectedly high activity in inhibiting the proteasome. In a preferred aspect of the invention, a pharmaceutical composition that includes a compound of Formula *VI* or Formula *VII* is administered to treat ischemic or

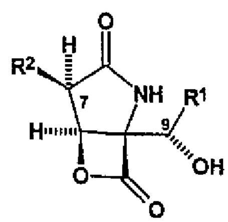
reperfusion injury. For example, in a preferred embodiment said compounds can be used to treat, prevent or ameliorate neuronal loss following stroke.

Synthetic Processes

A first aspect of the present invention relates to processes for forming lactacystin and analogs thereof having Formula ***VI*** and *clasto*-lactacystin β-lactone and analogs thereof having Formula ***VII***:



VI



VII

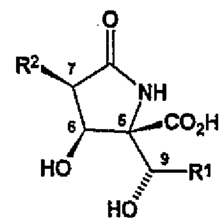
wherein

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^2 is alkyl, cycloalkyl, aryl, alkaryl, aralkyl, alkoxy, hydroxy, alkoxyalkyl, or amido, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted; and

R^7 is alkyl, aryl, aralkyl, alkaryl, wherein any of said alkyl, aryl, aralkyl or alkaryl can be optionally substituted.

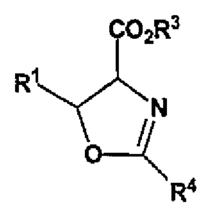
The processes for forming these compounds rely upon formation of a common carboxylic acid intermediate of Formula ***V***:



V

where R^1 and R^2 are as defined above for Formulae *VI* and *VII*. These steps include:

(a) deprotonating a substituted aryl or heteroaryl oxazoline of Formula *I*:

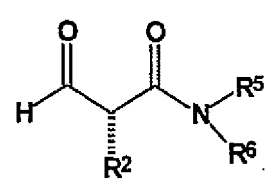


I

where R^1 is as defined above, and R^3 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^4 is aryl or heteroaryl, either of which may be optionally substituted; by treating said substituted aryl or heteroaryl oxazoline with a strong base to form an enolate;

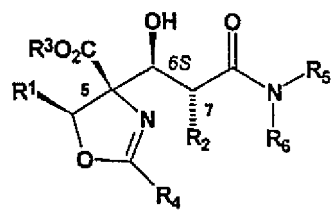
(b) transmetallating said enolate with a metal selected from the group consisting of titanium, aluminum, tin, zinc, magnesium and boron, and thereafter treating with a formyl amide of Formula *XIV*:



XIV

where R^2 is as defined above for Formulae *VI* and *VII*, and R^5 and R^6 are independently one of alkyl or alkaryl; or R^5 and R^6 when taken together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocyclic ring, which may be optionally substituted, and which optionally may include an additional oxygen or nitrogen atom,

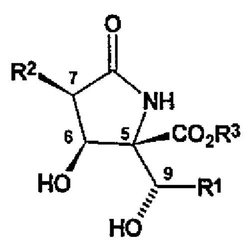
to form an adduct of Formula ***II***:



II

where R^1 through R^6 are as defined above;

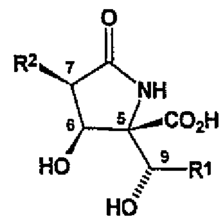
c) catalytically hydrogenating said adduct of Formula ***II*** to form a γ-lactam of Formula ***IV***:



IV

where R^1, R^2 and R^3 are as defined above;

d) saponifying said γ-lactam of Formula *IV* to form a lactam carboxylic acid of Formula *V*:



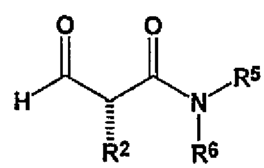
V

where R^1 and R^2 are as defined above.

The carboxylic acid intermediate of Formula *V* can be cyclized by treatment with a cyclizing reagent to form a *clasto*-lactacystin-β-lactone or analog thereof of Formula ***VII***, which can be optionally further reacted with a thiol (R^7SH), such as N-acetylcysteine, to form lactacystin or an analog thereof having Formula ***VI***.

Alternatively, the carboxylic acid intermediate of Formula *V* can be directly coupled to a thiol (R^7SH), such as N-acetylcysteine, to form lactacystin or an analog thereof having Formula ***VI***.

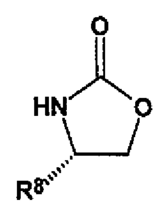
A second aspect of the present invention relates to the formation of enantiomerically-enriched formyl amides of Formula ***XIV***:



XIV

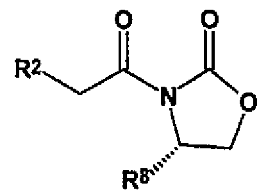
wherein R^2, R^5 and R^6 are as defined above, said method comprising:

(a) deprotonating a compound of Formula ***VIII:***



VIII

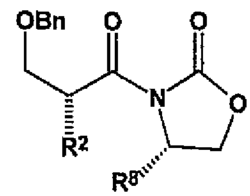
where R^8 is isopropyl or benzyl, and thereafter acylating the resultant anion with R^2CH_2COCl to form an acyloxazolidinone of Formula ***IX***:



IX

where R^2 and R^8 are as defined above;

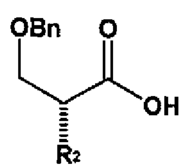
(b) stereoselectively reacting the acyloxazolidinone of Formula *IX* with benzyloxymethyl chloride to form a protected alcohol of Formula *X*:



X

where R^2 and R^8 are as defined above;

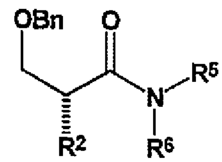
(c) hydrolyzing the protected alcohol of Formula *X* to form a carboxylic acid of Formula *XI*:



XI

where R^2 is as defined above;

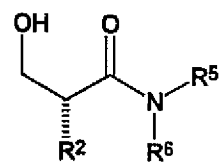
(d) coupling said acid of Formula *XI* with an amine $R^5R^6NH_2$ to provide an amide of Formula *XII*:



XII

where R^2, R^5 and R^6 are as defined above;

(e) catalytically hydrogenating, the amide of Formula *XII* to form an alcohol of Formula *XIII*:

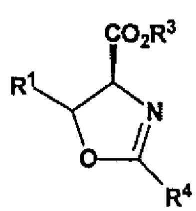


XIII

where R^2, R^5 and R^6 are as defined above; and

(f) oxidizing the resultant alcohol of Formula *XIII* to give a formyl amide of Formula *XIV*.

A third aspect of the invention relates to a process for forming a tri-substituted cis–oxazoline compound of Formula *Ia*:



Ia

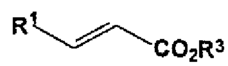
wherein

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

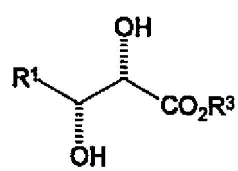
R^4 is aryl or heteroaryl, either of which may be optionally substituted; said method comprising:

(a) asymmetrically dihydroxylating an alkene intermediate of Formula ***XV***:



XV

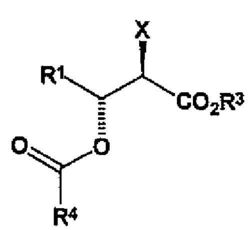
to form an optically active diol of Formula ***XVIa***:



XVIa;

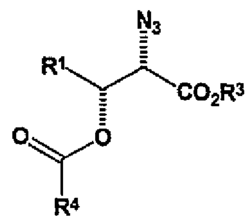
(b) reacting said optically active diol of Formula ***XVIa*** with an orthoester derived from an aromatic carboxylic acid under acid catalysis (Lewis or Brönsted acid) to give a mixed orthoester, and thereafter reacting the resulting mixed orthoester intermediate with a reagent selected from the group consisting of lower alkanoyl halides, hydrohalic acids (HX, where X is a halogen), acid chlorides, and halogen-containing Lewis acids (for example BBr_3, $SnCl_4$, $Ti(OR)_2Cl_2$, $Ti(OR)_3Cl$, Me_3SiX, where X is a halogen, and the like) in the presence of a base to form a derivative of Formula ***XVIIa***:

XVIIa;



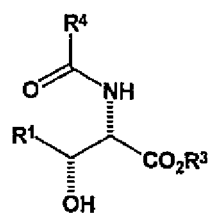
wherein X is a halogen, preferably Cl, Br or I;

(c) reacting said derivative of Formula ***XVIIa*** with an alkali metal azide to form an azide of Formula ***XVIIIa***:



XVIIIa;

(d) catalytically hydrogenating said azide to form a compound of Formula ***XIXa***:

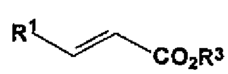


XIXa; and

(e) subjecting the compound of Formula ***XIXa*** to ring closing conditions to form said substituted aryl- or heteroaryloxazoline of Formula ***I*** with inversion of configuration at the oxygen-substituted carbon to produce a cis-oxazoline of Formula ***Ia***; wherein for each of Formulae ***XV***, ***XVIa***, ***XVIIa***, ***XVIIIa*** and ***XIXa***, R^1, R^3 and R^4 are as defined above for Formula ***I***.

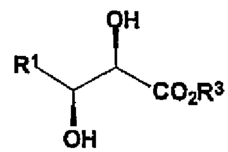
Alternatively, the third aspect of the invention relates to a process for forming a tri-substituted trans-oxazoline compound of Formula ***Ib*** comprising:

(a) asymmetrically dihydroxylating an alkene intermediate of Formula ***XV***:



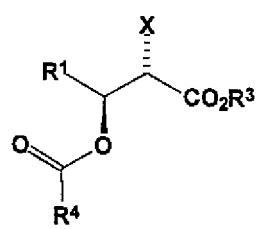
XV

to form an optically active diol of Formula ***XVIb***:



XVIb;

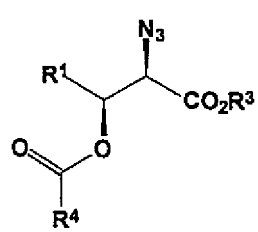
(b) reacting said optically active diol of Formula ***XVIb*** with an orthoester derived from an aromatic carboxylic acid under acid catalysis (Lewis or Brönsted acid) to give a mixed orthoester, and thereafter reacting the resulting mixed orthoester intermediate with a reagent selected from the group consisting of lower alkanoyl halides, hydrohalic acids (HX, where X is halogen), acid chlorides, and halogen-containing Lewis acids (for examples, BBr_3, $SnCl_4$, $Ti(OR)_2Cl_2$, $Ti(OR)_3Cl$, Me_3SiX, where X is a halogen, and the like) in the presence of a base to form a derivative of Formula ***XVIIb***:



XVIIb;

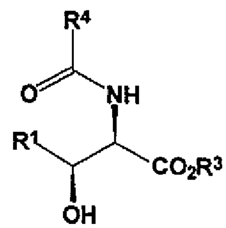
wherein X is a halogen, preferably Cl, Br, or I;

(c) reacting said derivative of Formula ***XVIIb*** with an alkali metal azide to form an azide of Formula ***XVIIIb***:



XVIIIb;

(d) catalytically hydrogenating said azide to form a compound of Formula ***XIXb***:



XIXb;

(e) subjecting the compound of Formula *XIXb* to ring closing conditions to form said substituted aryl- or heteroaryloxazoline of Formula ***Ib***, wherein the ring closure reaction proceeds with retention of configuration at the oxygen-substituted carbon to produce a *trans*-oxazoline of Formula ***Ib***; wherein for each of Formulae ***XV, XVI, XVII, XVIII*** and ***XIX***, R^1, R^3, and R^4 are as defined above for Formula ***I***.

With respect to the processes described above, the following preferred values are applicable:

Preferred values of R^1 are C_{1-12} alkyl, especially C_{1-8} alkyl, C_{3-8} cycloalkyl, especially C_{3-6} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, especially C_{6-10}aryl, C_{6-10}ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted. Substituents that can be optionally present on the aryl ring of an R^1 moiety include one or more, preferably one or two, of hydroxy, nitro, trifluoromethyl, halogen, C_{1-6} alkyl, C_{6-10} aryl, C_{1-6} alkoxy, C_{1-6} aminoalkyl, C_{1-6} aminoalkoxy, amino, C_{2-6} alkoxycarbonyl, carboxy, C_{1-6} hydroxyalkyl, C_{2-6} hydroxyalkoxy, C_{1-6} alkylsulfonyl, C_{6-10} arylsulfonyl, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonamido, C_{6-10} arylsulfonamido, C_{6-10} ar(C_{1-6}) alkylsulfonamido, C_{1-6} alkyl, C_{1-6} hydroxyalkyl, C_{6-10} aryl, C_{6-10} aryl(C_{1-6})alkyl, C_{1-6} alkylcarbonyl, C_{2-6} carboxyalkyl, cyano, and trifluoromethoxy.

R^1 is more preferably one of C_{1-8} alkyl such as ethyl, propyl or isopropyl; cycloalkyl, such as cyclohexyl; or C_{6-10} aryl, such as phenyl. Most preferred is isopropyl.

Preferred values of R^2 are C_{1-8} alkyl, C_{3-8} cycloalkyl, especially C_{3-6} cycloalkyl, C_{1-8} alkoxy, C_{2-8} alkenyl, C_{2-8} alkynyl C_{6-14} aryl, especially C_{6-10} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, where the ring portion of any of said aryl,

aralkyl, or alkaryl can be optionally substituted with any of the substituents as described for R^1 above.

R^2 is more preferably C_{1-4} alkyl, such as methyl, ethyl, propyl, or butyl; or C_{1-4} alkoxy, such as methoxy, or ethoxy. Most preferred are methyl, ethyl and propyl, and butyl.

With respect to R^3, a variety of ester functionalities can be employed at this position. Preferred values are C_{1-8} alkyl, C_{3-8} cycloalkyl, especially C_{4-7} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, especially C_{6-10} aryl, C_{6-10} ar(C_{1-6}) alkyl or C_{1-6}alk(C_{6-10})aryl, any of which can be optionally substituted. Substituents that can be optionally present on R^3 include one or more, preferably one or two, of the substituents as described for R^1 above.

R^3 is more preferably C_{1-4} alkyl, C_{6-10} aryl or C_{6-10} ar(C_{1-6})alkyl. Most preferred are methyl, ethyl, *tert*-butyl and benzyl.

R^4 is preferably C_{6-10} aryl, preferably phenyl, or a heteroaryl group selected from the group consisting of thienyl, benzo[b]thienyl, furyl, pyranyl, isobenzofuranyl, benzoxazolyl, 2*H*-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3*H*-indolyl, indolyl, indazolyl, purinyl, 4*H*-quinolizinyl, isoquinolyl, quinolyl, or triazolyl. The phenyl or heteroaryl group can be optionally substituted by one or two of the substituents as described for R^1 above. Most preferred are phenyl, and phenyl substituted by halogen, C_{1-6} alkyl, C_{1-6} alkoxy, carboxy, amino, C_{1-6} alkylamino and/or di(C_{1-6})alkylamino.

R^5 and R^6 are independently one of alkyl, aralkyl or alkaryl; or R^5 and R^6 when taken together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocyclic ring, which can be optionally substituted, and which optionally can include an additional oxygen or nitrogen atom. Optional substituents are those listed above for R^1.

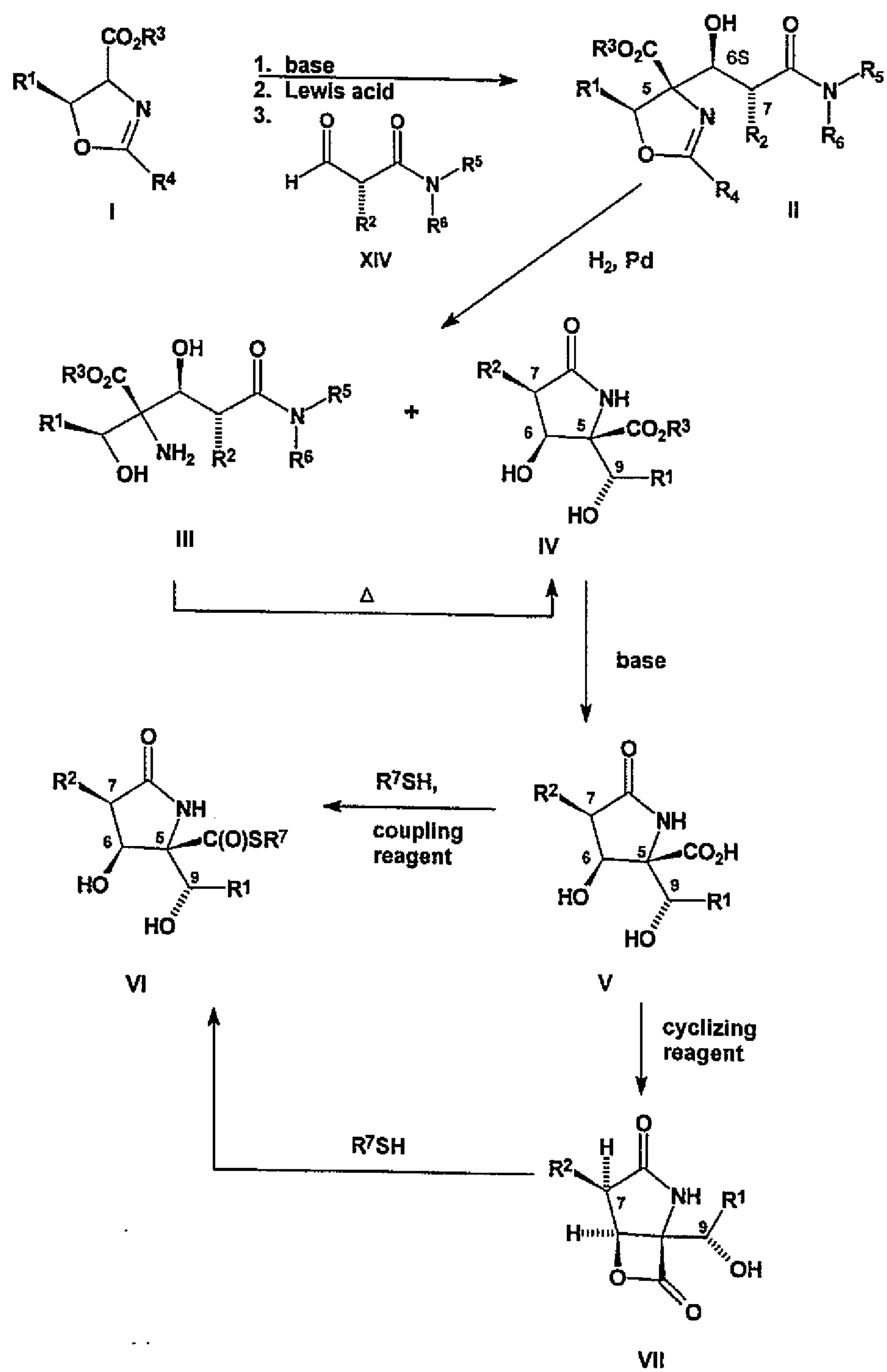
R^5 and R^6 are preferably C_{1-6} alkyl, C_{6-10}ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl or together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocycle which can be optionally substituted, and which optionally

can include an additional oxygen or nitrogen atom. Most preferred values for NR^5R^6 are dimethylamino, diethylamino, pyrrolidino, piperidino, morpholino, oxazolidinone, and oxazolidinone substituted by halogen, C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl, C_{1-6} alkoxy, carboxy, and/or amino.

R^7 is preferably C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{6-10} aryl, C_{6-10} ar(C_{1-6})alkyl, C_{1-6}alk(C_{6-10})aryl, any of which can be optionally substituted. Substituents that can be optionally present on either or both of the ring or chain portions of R^7 include one or more, preferably one or two, of the substituents as described for R^1 above. Preferably, R^7 together with the sulfur atom to which it is attached is cysteine or a derivative of cysteine such as N-acetyl cysteine, glutathione, and the like.

Scheme 1 is a general scheme for forming lactacystin and *clasto*-lactacystin-β-lactone analogs from substituted oxazoline starting materials.

Scheme 1

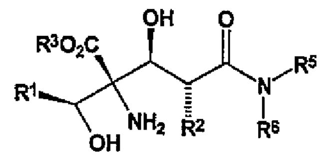


CO_2R^3
R^1
N
O
R^4
I
1. base
2. Lewis acid
3.
O
O
H
N
R^5
R^2
R^6
XIV
OH
O
R^3O_2C
6S
R_5
R^1
5
N
7
R_2
R_6
O
R_4
II
H_2, Pd
OH
O
R^3O_2C
R^5
R^1
NH_2
R^2
R^6
OH
III
+
O
R^2
7
NH
6
5
CO_2R^3
HO
9
R1
HO
IV
Δ
base
O
R^2
7
NH
6
5
C(O)SR7
HO
9
R1
HO
VI
R7SH,
coupling
reagent
O
R^2
7
NH
6
5
CO_2H
HO
9
R1
HO
V
cyclizing
reagent
R7SH
H
O
R^2
NH
R1
7
9
H
OH
O
O
VII

The starting oxazoline ***I***, which may be of either the *cis* (***Ia***) or *trans* (***Ib***) configuration, is deprotonated with a strong base to form the enolate. Examples of bases suitable for use in this reaction are organic bases, including hindered amide bases such as lithium diisopropylamide (LDA), lithium tetramethylpiperidide (LiTMP), lithium, sodium or potassium hexamethyldisilazide (LiHMDS, NaHMDS, KHMDS), or the like; or hindered alkyllithium reagents, such as *sec*-butyllithium, *tert*-butyllithium, or the like. The reaction is preferably conducted at reduced temperature in an ethereal solvent, such as diethyl ether, tetrahydrofuran (THF), or dimethoxyethane (DME). Reaction temperatures preferably range from about -100°C to about -30°C, more preferably from -85°C to -50°C, and most preferably from -85°C to -75°C. The reaction temperature is important in determining the stereochemical outcome of the subsequent addition to the aldehyde, with lower temperatures providing better selectivity.

The deprotonation step is followed by transmetallating said enolate with a metal selected from the group consisting of titanium, aluminum, tin, zinc, magnesium and boron. Preferred reagents for this step include titanium or aluminum Lewis acids, for example Me_2AlCl or $(i\text{-}PrO)_3TiCl$ or a mixture of the two. Preferably, between one and three molar equivalents of the Lewis acid are used, more preferably between two and three equivalents, and most preferably about 2.2-2.3 equivalents. Subsequent treatment of the enolate with a formyl amide (***XIV***) affords the adduct ***II***. Excess aldehyde is washed away with sodium bisulfite solution, and the crude material is carried forward to the next step without further purification. The use of 2.2-2.3 equivalents of Me_2AlCl results in selective formation of the (6*S*)-product (lactacystin numbering), in a ratio generally better than about 10:1, whereas the use of 1 equivalent of Me_2AlCl results in selective formation of the (6*R*)-product, in a ratio of about 5:1.

Catalytic hydrogenolysis of the adduct ***II***, as a mixture of (6*S*)- and (6*R*)-epimers, affords the desired γ-lactam (***IV***), sometimes as a mixture with the aminodiol ***III***:



III

Useful catalysts for this reaction include palladium black, palladium on activated carbon, palladium hydroxide on carbon, or the like. Organic solvents suitable for use in this reaction include lower alkanols such as methanol, ethanol, or isopropanol, lower alkanoates such as ethyl acetate, lower alkanoic acids such as acetic acid, or mixtures thereof. The reaction is conducted under an atmosphere of hydrogen, at pressures ranging from about 15 to about 100 p.s.i., more preferably from about 30 to about 50 p.s.i. Alternatively, transfer hydrogenation procedures (R.A.W. Johnstone *et al.*, *Chem. Rev. 85*:129 (1985)) may be used, in which the adduct ***II*** is treated at atmospheric pressure with a catalyst and a hydrogen donor.

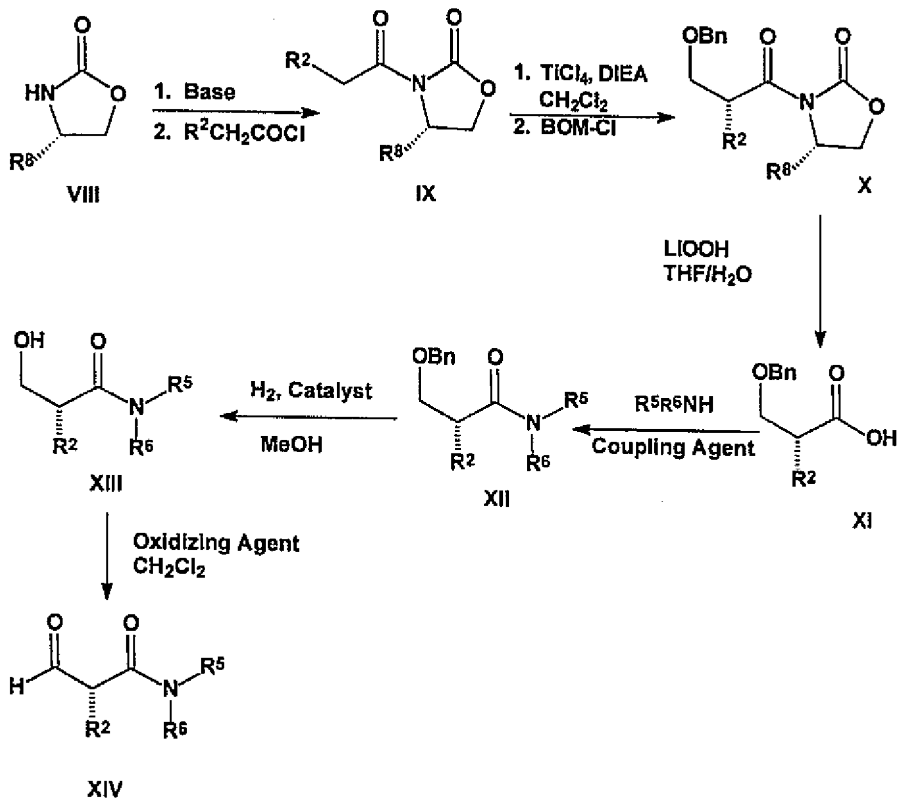
Upon heating of the crude product mixture, the aminodiol ***III*** is converted to the γ-lactam ***IV***, which can then be isolated in approximately 60-75% overall yield from ***II***. The heating step is conveniently carried out by first filtering off the catalyst used in the hydrogenation step and then heating the filtrate to reflux. When no aminodiol ***III*** is present in the crude product mixture, the heating step is omitted. Ester saponification, followed by cyclization, affords the β-lactone ***VII*** in 40-90% yield, and generally in greater than 60% yield. Cyclization can be effected with coupling reagents known in the art, including aryl sulfonyl chlorides, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent), *O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU), alkyl, aryl or alkenyl chloroformates, and the like. Isopropenyl chloroformate is a preferred reagent for this step, since all byproducts are volatile and chromatographic purification of the product is not necessary.

clasto-Lactacystin β-lactone can be converted to lactacystin by treatment of the β-lactone with *N*-acetylcysteine according to the reported procedure (Corey *et al., Tetrahedron Lett. 34*:6977 (1993)). Reactions of the β-lactone ***VII*** with

other thiols proceed analogously. Alternatively, lactacystin analogs are prepared by coupling the carboxylic acid intermediate *V* with a thiol to form the corresponding thiolester *VI*. The method of this invention is therefore useful for synthesis of both lactacystin and *clasto*-lactacystin β-lactone, as well as analogs thereof.

The enantiomerically-enriched formyl amides ***XIV*** employed in the aldol reaction are new. They can be prepared according to a representative reaction sequence such as that depicted in Scheme 2. For purposes of the present invention, the term "enantiomerically-enriched" means that one enantiomer is present in excess relative to the other; that is, one enantiomer represents greater than 50% of the mixture. The term "stereoselective" is used to mean that a synthesis or reaction step produces one enantiomer or diastereomer in excess relative to the other enantiomer or to other diastereomer(s).

Scheme 2

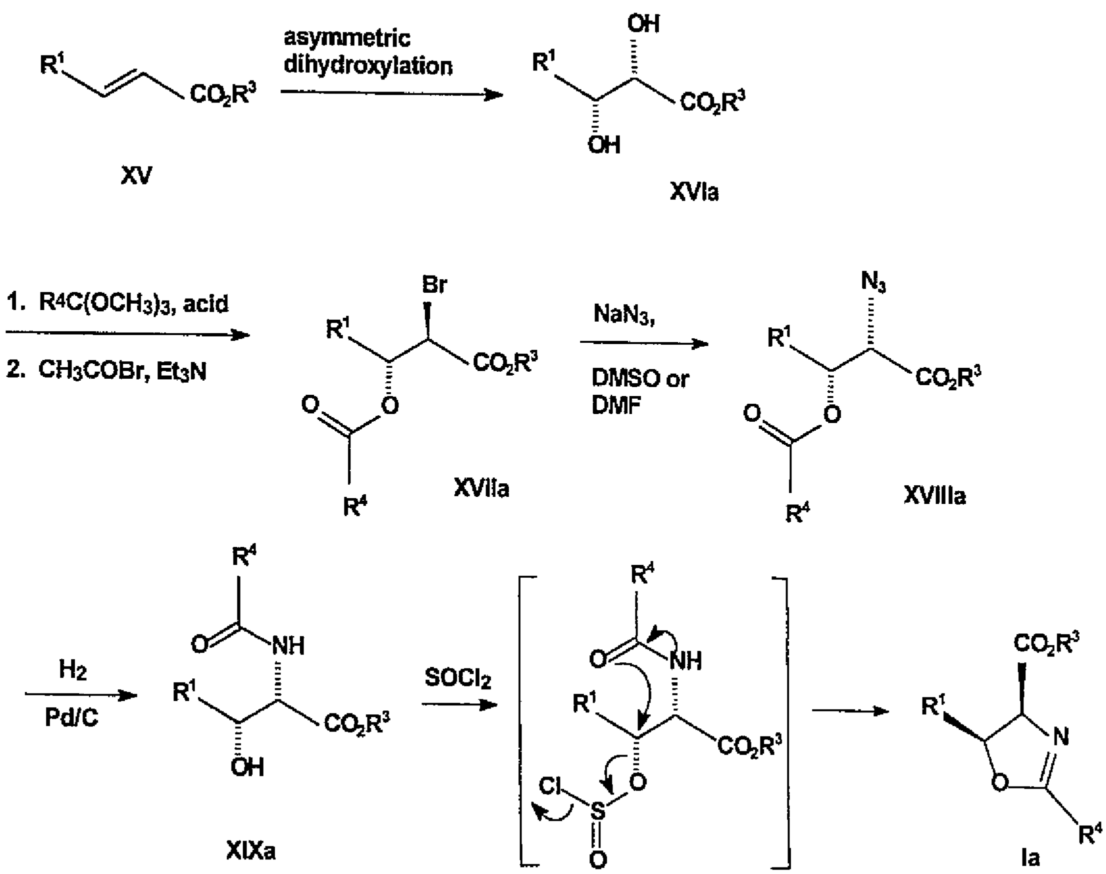


Acylation of the anion of (*S*)-(-)-4-benzyl-2-oxazolidinone (***VIIIa***) or (*S*)-(-)-4-isopropyl-2-oxazolidinone (***VIIIb***) (where R^8 is benzyl or isopropyl) affords the acyloxazolidinone ***IX*** in greater than 80% yield. Subsequent stereoselective benzyloxymethylation (Evans *et al., J. Am. Chem. Soc. 112*:8215 (1990)) gives the protected alcohol ***X*** in greater than 80% yield, provided that the benzyl chloromethyl ether is freshly prepared (Connor *et al., Organic Syntheses 52*:16 (1974)). Peroxide mediated hydrolysis affords the acid ***XI***, which is coupled with an amine to provide the amide ***XII***, generally in greater than 50% overall yield. Benzyl group hydrogenolysis, followed by oxidation of the resultant alcohol (***XIII***) then affords the formyl amide ***XIV*** in 80-85% yield. Pearlmans catalyst ($Pd(OH)_2$) is preferably used for the hydrogenolysis step. The final oxidation step is best

accomplished with the periodinane reported by Dess and Martin, *J. Org. Chem. 48*:4156 (1983) or with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) free radical, and buffered hypochlorite in the presence of bromide ion (*J. Org. Chem. 50*:4888 (1985); *Org. Synth. Coll. 8*:367 (1993)). Other mild oxidants such as tetrapropyl-ammonium perruthenate (TPAP) can also be used. The formyl amide ***XIV*** can be shown to be enantiomerically pure by reducing the aldehyde with sodium borohydride and converting the resultant alcohol to the corresponding Mosher ester using *R*-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (Dale *et al., J. Org. Chem. 34*:2543 (1969)). ^{1}H NMR analysis at 300 MHz reveals a single diastereomer. The aldehydes prepared according to Scheme 2 are configurationally stable, showing no signs of enantiomeric deterioration after one week, when stored at 0 °C. The aldehyde is also configurationally stable under the conditions of the aldol reaction, and the adduct ***II*** is formed without epimerization of the substituent R^2 at C(7).

The synthetic methods will work with any substituent at R^1 that is stable to strong base and to hydrogenation. Isopropyl is the preferred substituent for good proteasome inhibiting activity of the final product.

Scheme 3



The invention also relates to a new route to form the oxazoline starting material ***I***. The overall synthesis includes five steps (Scheme 3) and affords the *cis*-substituted oxazoline ***Ia***, which is thereafter employed in the method described above. The first step depicted in Scheme 3 is Sharpless asymmetric dihydroxylation (Sharpless *et al.*, *J. Org. Chem.* *57*:2768 (1992); Kolb *et al.*, *Chem. Rev.* *94*:2483 (1994); Shao and Goodman, *J. Org. Chem.* *61*:2582 (1996)) of the alkene ***XV***. If not commercially available, the alkene ***XV*** is prepared by Wittig condensation between the aldehyde and carboethoxymethylene triphenylphosphorane (Hale *et al.*, *Tetrahedron* *50*:9181 (1994)). Other

olefination procedures are also known in the art. The dihydroxylation reaction is preferably conducted with AD-mix-β (Aldrich Chemical Co.) in the presence of methane sulfonamide and stereoselectively affords the diol ***XVIa***, as predicted by the Sharpless face-selection rule. On a large scale, the dihydroxylation reaction is preferably conducted using N-methylmorpholine-N-oxide (NMO) as the reoxidant in place of $K_3Fe(CN)_6$ present in AD-mix-β. Although proceeding with somewhat lower enantioselectivity, this procedure allows more concentrated reaction mixtures and greatly simplifies the workup. The enantiomeric purity of the product can be enhanced by recrystallization.

In the next step, the diol ***XVIa*** is treated with an orthoester under Lewis or Brönsted acid catalysis to give a mixed orthoester, which is converted *in situ* to the haloester ***XVIIa*** by treatment with an acyl halide (Haddad *et al.*, *Tetrahedron Lett. 37*:4525 (1996)). Although acyl halides, especially acetyl halides are preferred for this reaction, other acid halides such as HCl, HBr, HI, Me_3SiCl, Me_3SiI, Me_3SiBr and the like may be used. Halogen-containing Lewis acids of the formula ML_nX, such as BBr_3, $SnCl_4$, $Ti(OR)_2Cl_2$, $Ti(OR)_3Cl$, and the like can also be used. In the previous formula, M is a metal selected from the group consisting of B, Ti, Sn, Al, Zn, and Mg; L is any suitable ligand for the metal, preferably an alkoxide or halogen group; n is an integer that results in a stable complex, and X is a halogen. Preferably acetyl bromide is used to produce the haloester ***XVIIa***. Preferably the orthoester employed in this reaction is derived from an aromatic or heteroaromatic carboxylic acid. More preferably, the orthoester is derived from benzoic acid, e.g., trimethyl orthobenzoate. The use of boron trifluoride etherate as the Lewis acid catalyst in the formation of the mixed orthoester is preferred, but other acids, such as HBr, $SnCl_4$, $TiCl_4$, BBr_3, and the like, can also be used.

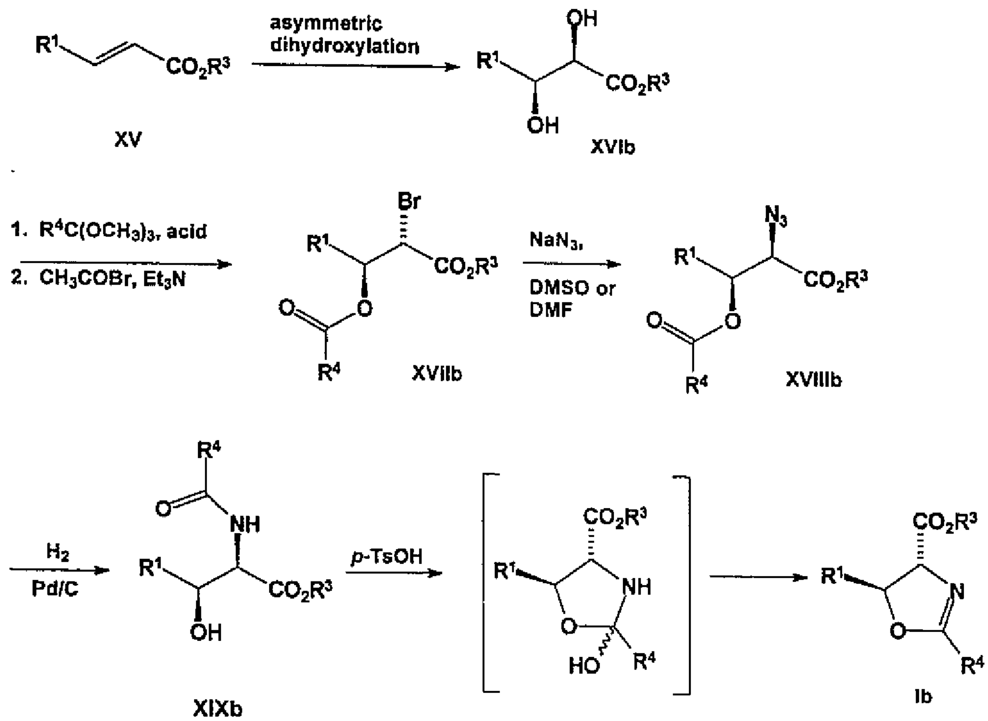
After workup, the crude halide ***XVIIa*** is converted to the azide ***XVIIIa*** by treatment with an alkali metal azide in a polar aprotic organic solvent, such as dimethyl sulfoxide (DMSO) or *N,N*-dimethyl formamide (DMF). Catalytic hydrogenation of the azide ***XVIIIa*** over a palladium catalyst in ethyl acetate

proceeds with concomitant migration of the aroyl group (Wang *et al.*, *J. Org. Chem.* *59*:5014 (1994)) to afford the hydroxyamide ***XIXa***.

Treatment of ***XIXa*** with thionyl chloride in methylene chloride effects ring closure with inversion of configuration at the hydroxyl-substituted carbon atom to produce the *cis*-substituted oxazoline starting material ***Ia***. Other reagents suitable for use in this reaction include sulfuryl chloride, phosphorous trichloride, phosphorous oxychloride, and (methoxycarbonylsulfamoyl)-triethylammonium hydroxide, inner salt (Burgess reagent). Treatment of **XIXa** under Mitsunobu conditions (Mitsunobu, *Synthesis:1* (1981) will also effect a ring closure. The oxazoline ring oxygen atom is destined to become the C(9)-hydroxyl group in the final products ***VI*** and ***VII***. Under equilibrating conditions (sodium methoxide, methanol), the *cis*-oxazoline (***Ia***) is converted to the *trans*-oxazoline (***Ib***) by inversion of configuration of the ester substituent, with the configuration of the R^1 substituent remaining fixed. The *cis*- and *trans*-oxazolines can both be used in the method depicted in Scheme 1, with equivalent results.

In an alternative route to form the oxazoline starting material ***I***, *p*-toluenesulfonic acid (*p*-TsOH) is used to effect ring closure (Scheme 4). In this case, ring closure proceeds with retention of configuration at the hydroxyl-substituted carbon atom to afford the *trans*-oxazoline (***Ib***). In order to obtain the proper stereochemistry at C(9) of the final product, the chiral ligand employed in the dihydroxylation reaction must be selected so as to provide the opposite face selectivity from that depicted in Scheme 3. For example, AD-mix-α is used in place of AD-mix-β. All other steps in the sequence proceed analogously to those described for the synthesis of the *cis*-oxazoline ***Ia***.

Scheme 4



Compounds

Many of the compounds described above are novel compounds; the novel compounds are also claimed.

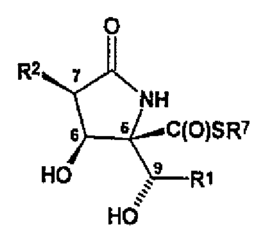
Fourth, fifth and sixth aspects of the invention relate to lactacystin analogs that can be made by the synthetic routes described herein; to pharmaceutical compositions including such compounds; and to methods of treating a subject having a condition mediated by proteins processed by the proteasome by administering to a subject an effective amount of a pharmaceutical composition disclosed herein. These methods include treatments for Alzheimers disease, cachexia, cancer, inflammation (e.g., inflammatory responses associated with

allergies, bone marrow or solid organ transplantation, or disease states, including but not limited to arthritis, multiple sclerosis, inflammatory bowel disease and parasitic diseases such as malaria), psoriasis, restenosis, stroke, and myocardial infarction.

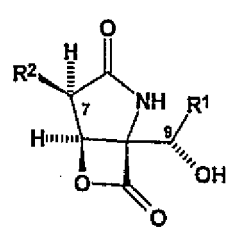
The compounds of Formulae *VI* and *VII* disclosed herein are highly selective for the proteasome, and do not inhibit other proteases such as trypsin, α-chymotrypsin, calpain I, calpain II, papain, and cathepsin B.

As disclosed by Fenteany *et al.* (WO 96/32105), hereby incorporated by reference in its entirety, lactacystin, *clasto*-lactacystin β-lactone, and analogs thereof possess biological activity as inhibitors of the proteasome. They can be used to treat conditions mediated directly by the function of the proteasome, such as muscle wasting, or mediated indirectly via proteins which are processed by the proteasome, such as the transcription factor NF-κB. The compounds prepared by the methods of this invention can also be used to determine whether a cellular, developmental, or physiological process or output is regulated by the proteolytic activity of the proteasome.

Those compounds that possess unexpected proteasome function-inhibiting activity are compounds of Formulae *VI* and *VII*:



VI



VII

or a salt thereof, wherein:

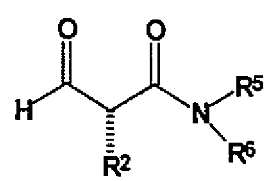
R^1 is C_{1-12} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl;

R^2 is C_{2-6} alkyl; and

R^7 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{6-10} aryl, C_{6-10} ar(C_{1-6})alkyl, C_{1-6}alk(C_{6-10})aryl, any of which can be optionally substituted. Substituents that can be optionally present on either or both of the ring or chain portions of R^7 include one or more, preferably one or two, of the substituents as described for R^1 above.

Preferred compounds are those where R^1 is C_{1-4} alkyl, more preferably isopropyl. R^2 is preferably ethyl, n-propyl, n-butyl or isobutyl. Preferably, R^7 together with the sulfur atom to which it is attached is cysteine or a derivative of cysteine such as N-acetyl cysteine, glutathione, and the like.

A seventh aspect of the present invention is directed to enantiomerically-enriched formyl amides of Formula ***XIV***:



XIV

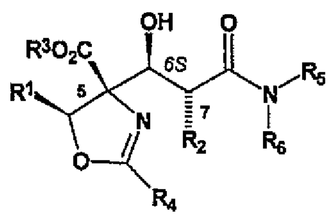
or salts thereof, wherein

R^2 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl; and

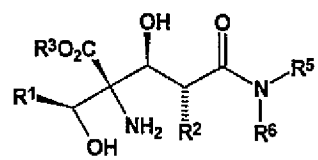
R^5 and R^6 are independently C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, or together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocycle which can be optionally substituted, and which optionally can include an additional oxygen or nitrogen atom.

Preferred compounds are those where R^2 is C_{2-6} alkyl.

An eighth aspect of the present invention is directed to compounds of Formulae ***II*** and ***III***:



II;



III

or salts thereof wherein

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^2 is alkyl, cycloalkyl, aryl, alkaryl, aralkyl, alkoxy, hydroxy, alkoxyalkyl, or amido, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted;

R^4 is optionally substituted aryl or optionally substituted heteroaryl; and

R^5 and R^6 are independently one of alkyl or alkaryl; or R^5 and R^6 when taken together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocyclic ring, which can be optionally substituted, and which optionally include an additional oxygen or nitrogen atom. Most preferred values for NR^5R^6 are dimethylamino, diethylamino, pyrrolidino, piperidino, morpholino, oxazolidinone, and oxazolidinone substituted by halogen, C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl, C_{1-6} alkoxy, carboxy, and/or amino.

Preferred compounds of Formulae *II* and *III* are those wherein:

R^1 is C_{1-12} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar (C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^2 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

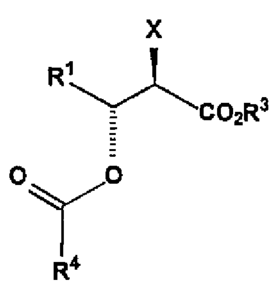
R^3 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, any of which can be optionally substituted;

R^4 is optionally substituted C_{6-10} aryl, or an optionally substituted heteroaryl group selected from the group consisting of thienyl, benzo[β]thienyl, furyl, pyranyl, isobenzofuranyl, benzoxazolyl, 2*H*-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3*H*-indolyl, indolyl, indazolyl, purinyl, 4*H*-quinolizinyl, isoquinolyl, quinolyl, or triazolyl; and

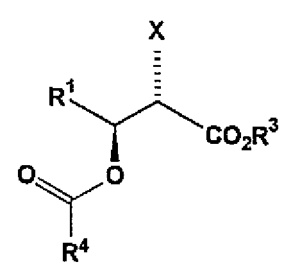
R^5 and R^6 are independently C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, or together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocycle which can be optionally substituted, and which optionally can include an additional oxygen or nitrogen atom. Most preferred values for NR^5R^6 are dimethylamino, diethylamino, pyrrolidino, piperidino, morpholino, oxazolidinone, and oxazolidinone substituted by halogen, C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl, C_{1-6} alkoxy, carboxy, and/or amino.

A ninth aspect of the present invention is directed to compounds of Formulae ***XVIIa***, ***XVIIb***, ***XVIIIa***, ***XVIIIb***, ***XIXa*** or ***XIXb***:

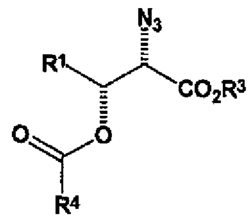
XVIIa



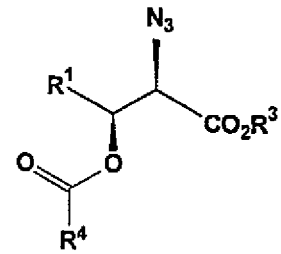
XVIIb



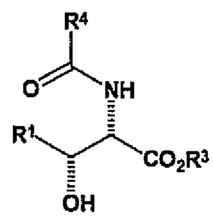
XVIIIa



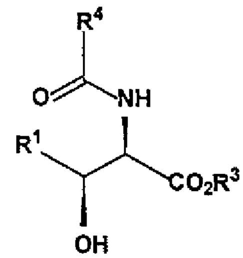
XVIIIb



XIXa



XIXb



or salts thereof, wherein

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^4 is optionally substituted aryl or optionally substituted heteroaryl.

Preferred compounds of Formulae *XVII*, *XVIII* or *XIX* are those wherein

R^1 is C_{1-12} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, any of which can be optionally substituted; and

R^4 is optionally substituted C_{6-10} aryl, or an optionally substituted heteroaryl group selected from the group consisting of thienyl, benzo[b]thienyl, furyl, pyranyl, isobenzofuranyl, benzoxazolyl, 2*H*-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl,

3*H*-indolyl, indolyl, indazolyl, purinyl, 4*H*-quinolizinyl, isoquinolyl, quinolyl, or triazolyl.

Definitions

The term "alkyl" as employed herein includes both straight and branched chain radicals of up to 12 carbons, preferably 1-8 carbons, such as methyl, ethyl, propyl, isopropyl, butyl, *t*-butyl, isobutyl, pentyl, hexyl, isohexyl, 1-ethylpropyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl and dodecyl.

The term "substituted alkyl" as employed herein, includes alkyl groups as defined above that have one, two or three halo, hydroxy, nitro, trifluoromethyl, halogen, C_{1-6} alkyl, C_{6-10} aryl, C_{1-6} alkoxy, C_{1-6} aminoalkyl, C_{1-6} aminoalkoxy, amino, C_{2-6} alkoxycarbonyl, carboxy, C_{1-6} hydroxyalkyl, C_{2-6} hydroxyalkoxy, C_{1-6} alkylsulfonyl, C_{6-10} arylsulfonyl, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonamido, C_{6-10} arylsulfonamido, C_{6-10} ar(C_{1-6}) alkylsulfonamido, C_{1-6} alkyl, C_{1-6} hydroxyalkyl, C_{6-10} aryl, C_{6-10} aryl(C_{1-6})alkyl, C_{1-6} alkylcarbonyl, C_{2-6} carboxyalkyl, cyano, and trifluoromethoxy and/or carboxy substituents.

The term "cycloalkyl" as employed herein includes saturated cyclic hydrocarbon groups containing 3 to 12 carbons, preferably 3 to 8 carbons, which include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl and cyclododecyl, any of which groups may be substituted with substituents such as halogen, C_{1-6} alkyl, C_{1-6} alkoxy and/or hydroxy group.

The term "heteroaryl" as employed herein refers to groups having 5 to 14 ring atoms, preferably 5, 6, 9 or 10 ring atoms; 6, 10 or 14 π electrons shared in a cyclic array; and containing carbon atoms and 1, 2 or 3 oxygen, nitrogen or sulfur heteroatoms (where examples of heteroaryl groups are: thienyl, benzo[b]thienyl, naphtho[2,3-b]thienyl, thianthrenyl, furyl, pyranyl, isobenzofuranyl, benzoxazolyl, chromenyl, xanthenyl, phenoxathiinyl, 2*H*-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl,

indolizinyl, isoindolyl, 3*H*-indolyl, indolyl, indazolyl, purinyl, 4*H*-quinolizinyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, tetrazolyl, quinazolinyl, cinnolinyl, pteridinyl, 4αH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, perimidinyl, phenanthrolinyl, phenazinyl, isothiazolyl, phenothiazinyl, isoxazolyl, furazanyl and phenoxazinyl groups).

The term "aryl" as employed herein by itself or as part of another group refers to monocyclic or bicyclic aromatic groups containing from 6 to 12 carbons in the ring portion, preferably 6-10 carbons in the ring portion, such as phenyl, naphthyl or tetrahydronaphthyl.

The term "aralkyl" or "arylalkyl" as employed herein by itself or as part of another group refers to C_{1-6} alkyl groups as discussed above having an aryl substituent, such as benzyl, phenylethyl or 2-naphthylmethyl.

The term "alkaryl" or "alkylaryl" as employed herein by itself or as part of another group refers to an aryl group as discussed above having a C_{1-6} alkyl substituent, such as toluyl, ethylphenyl, or methylnaphthyl.

The term "optionally substituted" when used with respect to aryl, aralkyl, alkaryl or 5-, 6-, 9- or 10- membered heteroaryl groups means that the ring portion of said groups can be optionally substituted by one or two substituents independently selected from C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{1-6} alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10}aryl, C_{6-10}aryl(C_{1-6})alkyl, C_{6-10}aryl(C_{1-6})alkoxy, hydroxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} arylthio, C_{6-10} arylsulfinyl, C_{6-10} arylsulfonyl, C_{6-10} aryl, C_{1-6} alkyl(C_{6-10})aryl, and halo(C_{6-10})aryl.

The term "alkoxy" refers to the above alkyl groups linked to oxygen.

The term "halogen" or "halo" as employed herein by itself or as part of another group refers to chlorine, bromine, fluorine or iodine.

The term "amido" as employed herein refers to formylamino, alkylcarbonylamino or arylcarbonylamino.

Uses

Pharmacological data for *clasto*-lactacystin β-lactone analogs prepared by the methods of this invention are provided in Table 1. These compounds are all irreversible inactivators of the 20S proteasome, acylating the *N*-terminal threonine residue of the X/MB1 subunit. The value $K_{obs}/[I]$ is a measure of the rate of enzyme inactivation. Several compounds show improved activity, i.e., more rapid rates of inactivation, when compared to *clasto*-lactacystin β-lactone itself (**2**). The compound that is most potent in the enzyme assay is the 7-methoxy derivative **3f**. However, when assayed in cell culture, **3f** is less potent than **2**.

The lactone ring is subject to nucleophilic attack not only by the threonine residue of the proteasome X/MB1 subunit, but also by water. Hydrolysis results in formation of the hydroxy acid **V**, which is not active as an inhibitor of the proteasome. Relative potency in cell culture is a composite of many factors, including enzyme potency, cell penetration, and hydrolysis rate. Although more potent than **2** against the enzyme, **3f** is also more rapidly hydrolyzed, resulting in much weaker activity in cell culture. By contrast, the analogs **3a-3d** show unexpectedly improved potency not only in the enzyme assay, but also in cell culture.

The disclosed compounds are used to treat conditions mediated directly by the proteolytic function of the proteasome such as muscle wasting, or mediated indirectly via proteins which are processed by the proteasome such as NF-κB. The proteasome participates in the rapid elimination and post-translational processing of proteins involved in cellular regulation (e.g., cell cycle, gene transcription, and metabolic pathways), intercellular communication, and the immune response (e.g., antigen presentation). Specific examples include β-amyloid protein and regulatory proteins such as cyclins and transcription factor NF-κB. Treating as used herein includes reversing, reducing, or arresting the symptoms, clinical signs, and underlying pathology of a condition in a manner to improve or stabilize the subject's condition.

Other embodiments of the invention relate to cachexia and muscle-wasting diseases. The proteasome degrades many proteins in maturing reticulocytes and growing fibroblasts. In cells deprived of insulin or serum, the rate of proteolysis nearly doubles.

Inhibiting the proteasome reduces proteolysis, thereby reducing both muscle protein loss and the nitrogenous load on kidneys or liver. Proteasome inhibitors are useful for treating conditions such as cancer, chronic infectious diseases, fever, muscle disuse (atrophy) and denervation, nerve injury, fasting, renal failure associated with acidosis, and hepatic failure. See, e.g., Goldberg, U.S. Pat. No. 5,340,736 (1994).

Embodiments of the invention therefore encompass methods for reducing the rate of muscle protein degradation in a cell, and reducing the rate of intracellular protein degradation. Each of these methods includes the step of contacting a cell (*in vivo* or *in vitro*, e.g., a muscle in a subject) with an effective amount of a compound (e.g., pharmaceutical composition) of a formula disclosed herein.

Proteasome inhibitors block processing of ubiquitinated NF-κB *in vitro* and *in vivo*. Proteasome inhibitors also block IκB-α degradation and NF-κB activation. (Palombella, *et al.*; and Traenckner, *et al.*, *EMBO J.* *13*:5433-5441 (1994)). One embodiment of the invention is a method for inhibiting IκB-α degradation, including contacting the cell with a compound of a formula described herein. A further embodiment is a method for reducing the cellular content of NF-κB in a cell, muscle, organ, or subject, including contacting the cell, muscle, organ, or subject with a compound of a formula described herein. Additional embodiments encompass methods for treating inflammatory responses associated with allergies, bone marrow or solid organ transplantation, or disease states, including but not limited to arthritis, inflammatory bowel disease, asthma, and multiple sclerosis by administering a compound of a formula disclosed herein. A preferred embodiment of the invention is directed to treating asthma by

administering a compound of Formula *VI* or Formula *VII*, most preferably compound ***3b***.

Proteasome inhibitors are also useful for treatment of ischemic or reperfusion injury, particularly for preventing or reducing the size of infarct after vascular occlusion such as occurs during a stroke or heart attack, as described in Brand, U.S. patent application Serial No. __________ (ProScript Docket No. 102.603.173), filed February 17, 1998, U.S. patent application Serial No. 08/988,339, filed December 3, 1997, and U.S. patent application Serial No. 08/801,936, filed February 15, 1998. Proteasome inhibitors also block proteasome-dependent transformation of protazoan parasites (Gonzalez *et al., J. Exp. Med. 184*:1909 (1996). Further embodiments of the invention therefore encompass methods for treating an infarct or a protazoan parasitic disease by administering a compound of a formula disclosed herein. In a preferred aspect of the invention, a compound of Formula *VI* or Formula *VII* is administered to prevent or reduce the size of the infarct after vascular occlusion. Said compounds can be administered from about 0 to about 10 hours after the occurrence of a stroke in order to treat or reduce neuronal loss following an ischemic event. Compounds ***3b*** is the most preferred compound in this aspect of the invention.

Proteasome inhibitors also block degradation of cell cycle regulatory proteins, such as cyclins and cyclin-dependent kinase inhibitors, and tumor suppressor proteins, such as p53. Other embodiments of the invention therefore encompass methods for blocking the cell cycle and for treating cell proliferative diseases such as cancer, psoriasis, and restenosis with a compound of a formula described herein.

The term "inhibitor" is meant to describe a compound that blocks or reduces the activity of an enzyme (e.g., the proteasome, or the X/MB1 subunit of the 20S proteasome). An inhibitor may act with competitive, uncompetitive, or noncompetitive inhibition. An inhibitor may bind reversibly or irreversibly, and therefore the term includes compounds which are suicide substrates of an enzyme.

An inhibitor may modify one or more sites on or near the active site of the enzyme, or it may cause a conformational change elsewhere on the enzyme.

Amounts and regimens for the administration of proteasome inhibitors and compositions of the invention can be determined readily by those with ordinary skill in the clinical art. Generally, the dosage of the composition of the invention will vary depending upon considerations such as: type of composition employed; age; health; medical conditions being treated; kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired; extent of tissue damage; gender; duration of the symptoms; and, counter indications, if any, and other variables to be adjusted by the individual physician. A desired dosage can be administered in one or more applications to obtain the desired results. Pharmaceutical compositions containing the proteasome inhibitors of the invention can be provided in unit dosage forms.

Compositions within the scope of this invention include all compositions wherein the compounds of the present invention are contained in an amount which is effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typically, the compounds may be administered to mammals, e.g. humans, orally at a dose of 0.0025 to 50 mg/kg, or an equivalent amount of the pharmaceutically acceptable salt thereof, per day of the body weight of the mammal being treated for a proteosome-mediated condition such as a stroke or asthma. For intramuscular injection, the dose is generally about one-half of the oral dose.

In the method of prevention or reduction of infarct size the compound can be administered by intravenous injection at a dose of about 0.01 to about 10 mg/kg, preferably about 0.025 to about 1 mg/kg.

The unit oral dose may comprise from about 0.01 to about 50 mg, preferably about 0.1 to about 10 mg of the compound. The unit dose may be administered one or more times daily as one or more tablets each containing from about 0.1 to about 10, conveniently about 0.25 to 50 mg of the compound or its

solvates. For use in treating stroke, it is preferred that a single dosage be administered, 0 to about 10 hours post-event, preferably 0 to about 6 hours post-event.

The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered and obvious to those skilled in the art are within the spirit and scope of the invention.

The preparation of formyl amides XIV according to the synthetic scheme depicted in scheme 2 as exemplified in Examples 1-6.

Example 1: Acyl Oxazolidinones (IX)

a. **Acyl oxazolidinone IXb (R^2 = *n*-Pr; R^8 = CH_2Ph):** A cooled (-78°C) solution of (*S*)-(-)-4-benzyl-2-oxazolidinone (4.0 g, 22.6 mmol) in 75 mL anhydrous THF was treated with a 2.5 M solution of n-BuLi in hexane (9.1 mL, 22.6 mmol) over 15 min. After 5 min, neat valeryl chloride (2.95 mL, 24.9 mmol) was added dropwise and the mixture was stirred for another 45 min. at -78°C. The mixture was then allowed to reach room temperature, stirred for another 90 min, and then treated with 50 mL saturated NH_4Cl solution. Dichloromethane (50 mL) was then added and the organic layer was washed with brine (2 x 30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. This afforded 5.94 g (100 %) of the desired acyl oxazolidinone **IXb** as a clear colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.36-7.20 (m, 5H), 4.71-4.64 (m, 1H), 4.23-4.14 (m, 1H), 3.40 (dd, *J*= 13.3, 3.2 Hz, 1H), 3.04-2.84 (m, 2H), 2.77 (dd, *J*=13.3, 9.6 Hz, 1H), 1.74-1.63 (m, 2H), 1.46-1.38 (m, 2H), 0.96 (t, *J*=7.3 Hz, 3H).

b. **Acyl oxazolidinone IXa (R^2 = Et; R^8 = CH_2Ph):** By a procedure analogous to that described for preparing acyl oxazolidinone **IXb**, the lithium anion of (*S*)-(-)-4-benzyl-2-oxazolidinone was treated with butyryl chloride to provide acyl oxazolidinone **IXa** in 94% yield. ^1H NMR (300 MHz, $CDCl_3$) δ

7.37-7.20 (m, 5H), 4.68 (ddd, J= 13.1, 7.0, 3.4 Hz, 1H), 4.23-4.13 (m, 2H), 3.30 (dd, J= 13.3, 9.6 Hz, 1H), 3.02-2.82 (m, 2H), 2.77 (dd, J=13.3, 9.6 Hz, 1H), 1.73 (q, J= 7.3 Hz, 2H), 1.01 (t, J= 7.3 Hz, 3H).

c. Acyl oxazolidinone IXc (R^2 = *n*-Bu; R^8 = CH_2Ph): By a procedure analogous to that described for preparing acyl oxazolidinone **IXb**, the lithium anion of (*S*)-(-)-4-benzyl-2-oxazolidinone was treated with hexanoyl chloride to provide acyl oxazolidinone **IXc** in 96% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 7.36-7.20 (m, 5H), 4.68 (m, 1H), 4.23-4.14 (m, 2H), 3.30 (dd, J= 13.3, 3.3 Hz, 1H), 3.02-2.83 (m, 2H), 2.76 (dd, J=13.3, 9.6 Hz, 1H), 1.70 (m, 2H), 1.43-1.34 (m, 4H), 0.92 (t, J= 3.3 Hz, 3H).

d. Acyl oxazolidinone IXd (R^2 = *i*-Bu; R^8 = CH_2Ph):

i. 4-Methylvaleryl chloride

4- Methylvaleryl chloride was prepared from commercially available 4-methylvaleric acid in the following way: a cold (0 °C) solution of 4-methylvaleric acid (1.85 mL, 15.0 mmol) in 50 mL anhydrous CH_2Cl_2 containing 10 mL of DMF was treated with 1.95 μL oxalyl chloride (22.5 mmol). The mixture was then stirred for 3 h at room temperature, concentrated *in vacuo* and filtered to affords 1.65 g (100%) of the desired acid chloride as a colorless liquid.

ii. Acyl oxazolidinone IXd (R^2 = *i*-Bu; R^8 = CH_2Ph):

By a procedure analogous to that described for preparing acyl oxazolidinone **IXb**, the lithium anion of (*S*)-(-)-4-benzyl-2-oxazolidinone was treated with 4-methylvaleryl chloride to provide acyl oxazolidinone **IXd** in 85% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 7.37-7.20 (m, 5H), 4.70-4.63 (m, 1H), 4.23-4.15 (m, 2H), 3.30 (dd, J= 13.2, 3.2 Hz, 1H), 2.98-2.90 (m, 2H), 2.76 (dd, J=13.3, 9.6 Hz, 1H), 1.68-1.54 (m, 3H), 0.94 (d, J= 6.2 Hz, 3H).

e. Acyl oxazolidinone IXe (R^2 = CH_2Ph; R^8 = CH_2Ph): By a procedure analogous to that described for preparing acyl oxazolidinone **IXb**, the lithium anion of (*S*)-(-)-4-benzyl-2-oxazolidinone was treated with hydrocinnamoyl chloride to provide acyl oxazolidinone **IXe** in 82% yield. ^1H NMR (300 MHz,

$CDCl_3$) δ 7.35-7.16 (m, 10H), 4.70-4.63 (m, 1H), 4.21-4.14 (m, 2H), 3.38-3.19 (m, 3H), 3.08-2.98 (m, 2H), 2.75 (dd, *J*=13.4, 9.5 Hz, 1H).

Example 2: Acyl Oxazolidinones (X)

a. **Acyl oxazolidinone Xb (R^2 = *n*-Pr; R^8 = CH_2Ph):** A cold (0°C) solution of acyl oxazolidinone **IXb** (5.74 g, 22.0 mmol) in 110 mL anhydrous CH_2Cl_2 was treated with 2.52 mL $TiCl_4$ (23.1 mmol) resulting in the formation of an abundant precipitate. After 5 min, diisopropylethylamine (4.22 mL, 24.2 mmol) was added slowly and the resulting dark brown solution was stirred at room temperature for 35 min. Benzyl chloromethyl ether (6.0 mL, 44.0 mmol) was rapidly added and the mixture was stirred for 5 h at room temperature. 50 mL CH_2Cl_2 and 75 mL of 10% aqueous NH_4Cl were then added, resulting in the formation of yellow gummy material. After stirring the suspension vigorously for 10 min, the supernatant was transferred in a separatory funnel and the gummy residue was taken up in 100 mL 1:1 10% aqueous NH_4Cl/CH_2Cl_2. The combined organic layers were then washed successively with 1N aqueous HCl, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude solid material was recrystallized from EtOAc/hexane affording 6.80 g of desired acyl oxazolidinone **Xb** as a white solid in 81% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 7.34-7.18 (m, 10H), 4.77-4.69 (m, 1H), 4.55 (s, 2H), 4.32-4.23 (m, 1H), 4.21-4.10 (m, 2H), 3.80 (t, *J*= 9.0 Hz, 1H), 3.65 (dd, *J*= 9.0, 5.0 Hz, 1H), 3.23 (dd, *J*= 13.5, 3.3 Hz, 1H), 2.69 (dd, *J*= 13.5, 9.3 Hz, 1H), 1.74-1.64 (m, 1H), 1.54-1.44 (m, 1H), 1.40-1.28 (m, 2H), 0.91 (t, *J*= 7.3 Hz, 3H).

LRMS (FAB) *m/e* 382 ($M+H^+$)

b. **Acyl oxazolidinone Xa (R^2 = Et; R^8 = CH_2Ph):** By a procedure analogous to that described for preparing acyl oxazolidinone **Xb**, acyl oxazolidinone **Xa** was obtained in 80% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 7.36-7.18 (m, 10H), 4.55 (s, 2H), 4.21-4.11 (m, 3H), 3.81 (t, *J*= 9.0 Hz, 1H),

3.66 (dd, J= 9.0, 5.0 Hz, 1H), 3.23 (dd, J= 13.5, 3.2 Hz, 1H), 2.70 (dd, J= 13.5, 9.3 Hz, 1H), 1.78-1.57 (m, 2H), 0.94 (t, J= 7.5 Hz, 3H).

c. **Acyl oxazolidinone Xc (R^2 = *n*-Bu; R^8 = CH_2Ph):** By a procedure analogous to that described for preparing acyl oxazolidinone **Xb**, acyl oxazolidinone **Xc** was obtained in 91% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.17 (m, 10H), 4.72 (m, 1H), 4.54 (s, 2H), 4.27-4.10 (m, 2H), 3.79 (t, J= 8.7 Hz, 1H), 3.65 (dd, J= 9.1, 5.0 Hz, 1H), 3.23 (dd, J= 13.5, 3.3 Hz, 1H), 2.68 (dd, J= 13.5, 9.3 Hz, 1H), 1.75-1.68 (m, 1H), 1.31-1.26 (m, 4H), 0.87 (t, J= 6.8 Hz, 3H).

d. **Acyl oxazolidinone Xd (R^2 = *i*-Bu; R^8 = CH_2Ph):** By a procedure analogous to that described for preparing acyl oxazolidinone **Xb**, acyl oxazolidinone **Xd** was obtained in 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.17 (m, 10H), 4.75-4.67 (m, 1H), 4.57 (d, J= 12.0 Hz, 1H), 4.51 (d, J= 12.0 Hz, 1H), 4.41-4.36 (m, 1H), 4.20-4.09 (m, 2H), 3.74 (t, J= 9.0 Hz, 1H), 3.65 (dd, J= 9.0, 5.1 Hz, 1H), 3.23 (dd, J= 13.5, 3.2 Hz, 1H), 2.63 (dd, J= 13.5, 9.5 Hz, 1H), 1.74-1.52 (m, 2H), 1.35 (dd, J= 13.1, 6.1 Hz, 1H), 0.92 (d, J= 2.9 Hz, 3H), 0.90 (d, J= 2.9 Hz, 3H).

e. **Acyl oxazolidinone Xe (R^2 = CH_2Ph; R^8 = CH_2Ph):** By a procedure analogous to that described for preparing acyl oxazolidinone **Xb**, acyl oxazolidinone **Xe** was obtained in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.15 (m, 15H), 4.62-4.50 (m, 4H), 4.03 (dd, J= 9.0, 2.7 Hz, 1H), 3.93-3.82 (m, 2H), 3.66 (dd, J= 9.2, 4.8 Hz, 1H), 3.19 (dd, J= 13.5, 3.2 Hz, 1H), 2.98 (dd, J= 13.4, 8.2 Hz, 1H), 2.88 (dd, J= 13.4, 7.3 Hz, 1H), 2.68 (dd, J= 13.5, 9.3 Hz, 1H).

Example 3: Carboxylic Acids (XI)

a. **Carboxylic acid XIb (R^2 = *n*-Pr):** A cold (0°C) solution of 6.60 g (17.3 mmol) of acyl oxazolidinone **Xb** in 320 mL THF/H_2O was treated successively with 6.95 mL 35% aqueous H_2O_2 and a solution of lithium hydroxide

monohydrate (1.46 g, 34.6 mmol) in 20 mL H_2O. The mixture was stirred for 16 h at 0°C and then treated carefully first with a solution Na_2SO_3 (10.5 g) in 55 mL H_2O and then with a solution of $NaHCO_3$ (4.35 g) in 100 mL H_2O. The mixture was stirred for 30 min at room temperature and concentrated *in vacuo* to remove the THF. The resulting aqueous mixture was then washed with CH_2Cl_2 (4 x 75 mL), cooled to 0°C, acidified with 6N aqueous HCl and extracted with CH_2Cl_2 (1 x 200 mL and 3 x 100 mL). The combined organic layers were then dried over $MgSO_4$ and concentrated in vacuo affording 3.47 g (90%) of desired acid **XIb** as a clear colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.26 (m, 5H), 4.55 (s, 2H), 3.67 (m, 1H), 3.57 (dd, *J*= 9.2, 5.2 Hz, 1H), 2.75 (m, 1H), 1.72-1.31 (m, 4H), 0.93 (t, *J*= 7.2 Hz, 3H). LRMS (FAB) *m/e* 223 (M+H$^+$)

b. **Carboxylic acid XIa (R^2 = Et):** By a procedure analogous to that described for preparing acyl oxazolidinone **XIb**, acyl oxazolidinone **XIa** was obtained in 48% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.27 (m, 5H), 4.55 (s, 2H), 3.68 (dd, *J*= 9.2, 7.9 Hz, 1H), 3.59 (dd, *J*= 9.2, 5.4 Hz, 1H), 2.68-2.65 (m, 1H), 1.71-1.62 (m, 2H), 0.97 (t, *J*= 7.5 Hz, 3H).

c. **Carboxylic acid XIc (R^2 = *n*-Bu):** By a procedure analogous to that described for preparing acyl oxazolidinone **XIb**, acyl oxazolidinone **XIc** was obtained in 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37-7.28 (m, 5H), 4.55 (s, 2H), 3.67 (dd, *J*= 9.1, 8.1 Hz, 1H), 3.57 (dd, *J*= 9.2, 5.3 Hz, 1H), 2.72 (m, 1H), 1.67-1.51 (m, 2H), 1.36-1.27 (m, 4H), 0.89 (t, *J*= 6.9 Hz, 3H).

d. **Carboxylic acid XId (R^2 = *i*-Bu):** By a procedure analogous to that described for preparing acyl oxazolidinone **XIb**, acyl oxazolidinone **XId** was obtained in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37-7.28 (m, 5H), 4.55 (s, 2H), 3.64 (t, *J*= 9.1 Hz, 1H), 3.54 (dd, *J*= 9.1, 5.1 Hz, 1H), 2.81 (m, 1H), 1.68-1.54 (m, 2H), 1.36-1.27 (m, 1H), 0.92 (d, *J*= 4.9 Hz, 3H), 0.90 (d, *J*= 4.9 Hz, 3H).

e. **Carboxylic acid XIe (R^2 = CH_2Ph):** By a procedure analogous to that described for preparing acyl oxazolidinone **XIb**, acyl oxazolidinone **XIe** was obtained in 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.16 (m, 10H), 4.53

(d, J= 12.1 Hz, 1H), 4.50 (d, J= 12.1 Hz, 1H), 3.68-3.57 (m, 2H), 3.09-2.85 (m, 3H).

Example 4: Diethyl Amides (XII)

a. **Diethylamide XIIb (R^2 = *n*-Pr; R^5 = R^6 = Et):** A cooled solution (0°C) of carboxylic acid **XIb** (3.40 g, 15.3 mmol) in 1:1 MeCN/CH_2Cl_2 (150 mL), containing diethylamine (2.36 mL, 23.0 mmol) and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 5.89 g, 18.4 mmol), was treated with diisopropylethylamine (6.7 mL, 38.2 mmol) over 1.5 h (syringe pump). The mixture was then concentrated in vacuo and partitioned between ether (200 mL) and H_2O (100 mL). The aqueous layer was extracted with more ether (2 x 100 mL) and the combined organic layers were washed with aqueous 1N HCl (3 x 50 mL), saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Chromatographic purification (230-400 mesh SiO_2, elution with 1:3 AcOEt/hexane) afforded 4.24 g (97%) of diethyl amide **XIIb** as a clear colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.35-7.23 (m, 5H), 4.52 (d, J= 12.0 Hz, 1H), 4.44 (d, J= 12.0 Hz, 1H), 3.67 (t, J= 8.6 Hz, 1H), 3.51 (dd, J= 8.7, 5.5 Hz, 1H), 3.46-3.27 (m, 4H), 2.96 (m, 1H), 1.67-1.57 (m, 1H), 1.48-1.22 (m, 4H), 1.20-1.10 (m, 6H), 0.90 (t, J= 7.2 Hz, 3H). LRMS (FAB) *m/e* 278 ($M+H^+$)

b. **Diethylamide XIIa (R^2 = Et; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing diethylamide **XIIb**, diethylamide **XIIa** was obtained in 73% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 7.33-7.26 (m, 5H), 4.52 (d, J= 12.0 Hz, 1H), 4.44 (d, J= 12.0 Hz, 1H), 3.68 (t, J= 8.6 Hz, 1H), 3.53-3.33 (m, 5H), 2.90 (m, 1H), 1.75-1.50 (m, 2H), 1.18 (t, J= 7.1 Hz, 3H), 1.13 (t, J= 7.1 Hz, 3H), 0.89 (t, J= 7.4 Hz, 3H).

c. **Diethylamide XIIc (R^2 = *n*-Bu; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing diethylamide **XIIb**, diethylamide **XIIc** was obtained in 94% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 7.35-7.25 (m, 5H),

4.51 (d, J= 12.0 Hz, 1H), 4.44 (d, J= 12.0 Hz, 1H), 3.67 (t, J= 8.6 Hz, 1H), 3.51 (dd, J= 8.8, 5.5 Hz, 1H), 3.46-3.29 (m, 1H), 2.94 (m, 1H), 1.66-1.62 (m, 2H), 1.33-1.10 (m, 9H), 0.85 (t, J= 7.0 Hz, 3H).

d. **Diethylamide XIId (R^2 = *i*-Bu; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing diethylamide **XIIb**, diethylamide **XIId** was obtained in 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.23 (m, 5H), 4.51 (d, J= 12.0 Hz, 1H), 4.44 (d, J= 12.0 Hz, 1H), 3.65 (t, J= 8.7 Hz, 1H), 3.54-3.28 (m, 5H), 3.03 (m, 1H), 1.63-1.49 (m, 2H), 1.33-1.24 (m, 1H), 1.18 (t, J= 7.1 Hz, 3H), 1.12 (t, J= 7.1 Hz, 3H), 0.90 (t, J= 6.4 Hz, 3H).

e. **Diethylamide XIIe (R^2 = CH_2Ph; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing diethylamide **XIIb**, diethylamide **XIIe** was obtained in 89% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.16 (m, 10H), 4.53 (d, J= 12.1 Hz, 1H), 4.47 (d, J= 12.1 Hz, 1H), 3.77 (t, J= 8.5 Hz, 1H), 3.59 (dd, J= 8.8, 5.7 Hz, 1H), 3.40 (m, 1H), 3.22-2.89 (m, 5H), 2.79 (dd, J= 13.0, 5.1 Hz, 3H), 1.01 (t, J= 7.1 Hz, 3H), 0.85 (t, J= 7.2 Hz, 3H).

Example 5: Alcohols (XIII)

a. **Alcohol XIIIb (R^2 = *n*-Pr; R^5 = R^6 = Et):** To a solution of diethylamide **XIIb** (4.08 g, 14.7 mmol) in 140 mL MeOH was added 20% $Pd(OH)_2/C$ (400 mg) and the suspension was hydrogenated at atmospheric pressure and room temperature for 15 h. Filtration of the catalyst and concentrating the filtrate in vacuo afforded 2.84 g (100%) of the desired primary alcohol **XIIIb**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (br. d, J=4.2 Hz, 1H), 3.61-3.15 (m, 5H), 2.71 (m, 1H), 1.69-1.24 (m, 4H), 1.20 (t, J= 7.1 Hz, 3H), 1.12 (t, J= 7.1 Hz, 3H), 0.92 (t, J= 7.2 Hz, 3H). LRMS (FAB) *m/e* 188 ($M+H^+$).

b. **Alcohol XIIIa (R^2 = Et; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing alcohol **XIIIb**, alcohol **XIIIa** was obtained in 100% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76 (m, 2H), 3.58-3.19 (m, 4H), 2.64 (m, 1H),

1.71-1.65 (m, 2H), 1.21 (t, J= 7.1 Hz, 3H), 1.13 (t, J= 7.1 Hz, 3H), 0.96 (t, J= 7.4 Hz, 3H).

c. **Alcohol XIIIc (R^2 = *n*-Bu; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing alcohol **XIIIb**, alcohol **XIIIc** was obtained in 100% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76 (d, J= 4.5 Hz, 2H), 3.58-3.19 (m, 4H), 2.72-2.65 (m, 2H), 1.68-1.55 (m, 2H), 1.40-1.24 (m, 4H), 1.20 (t, J= 7.1 Hz, 3H), 1.12 (t, J= 7.1 Hz, 3H), 0.90 (t, J= 6.9 Hz, 3H).

d. **Alcohol XIIId (R^2 = *i*-Bu; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing alcohol **XIIIb**, alcohol **XIIId** was obtained in 100% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78-3.68 (m, 2H), 3.57-3.15 (m, 4H), 2.81-2.73 (m, 1H), 1.70-1.60 (m, 2H), 1.40-1.28 (m, 1H), 1.21 (t, J= 7.1 Hz, 3H), 1.12 (t, J= 7.1 Hz, 3H), 0.92 (m, 6H).

e. **Alcohol XIIIe (R^2 = CH_2Ph; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing alcohol **XIIIb**, alcohol **XIIIe** was obtained in 100% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29-7.16 (m, 5H), 3.81-3.71 (m, 2H), 3.61-3.50 (m, 1H), 3.15-2.87 (m, 6H), 1.05 (t, J= 7.1 Hz, 3H), 0.98 (t, J= 7.1 Hz, 3H).

Example 6: Aldehydes (XIV)

a. **Aldehyde XIVb (R^2 = *n*-Pr; R^5 = R^6 = Et):** To a solution of alcohol **XIIIb** (2.34 g, 12.7 mmol) in wet CH_2Cl_2 (125 mL, prepared by stirring CH_2Cl_2 with water and separating the organic layer) was added Dess-Martin periodinane (8.06 g, 19.0 mmol). The mixture was stirred at room temperature for 40 min and was then poured into a mixture of 5% aqueous $Na_2S_2O_3$ (250 mL) containing 5.2 g $NaHCO_3$, and ether (200 mL). The biphasic mixture was stirred vigorously for 5 min and the aqueous layer was extracted with 15% CH_2Cl_2/Et_2O (2 x 100 mL). The combined organic layers were then washed with H_2O (3 x 75 mL) and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 2.06 g (88%) of desired aldehyde **XIVb**, a clear colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.60

(d, J= 3.5 Hz, 1H), 3.49-3.30 (m, 5H), 1.96-1.85 (m, 2H), 1.39-1.31 (m, 2H), 1.19 (t, J=7.1 Hz, 3H), 1.13 (t, J= 7.1 Hz, 3H), 0.95 (t, J= 7.3 Hz, 3H).

b. **Aldehyde XIVb (R^2 = *n*-Pr; R^5 = R^6 = Et):** To a solution of crude **XIIIb** (1.25 g, 6.68 mmol) in a mixture of toluene (20 mL), ethyl acetate (20 mL), and water (3 mL) was added 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO), free radical (9 mg). The mixture was cooled to 0°C and a sodium hypochlorite solution, prepared by adding 4.3 mL of aqueous sodium hypochlorite (10-13% available chlorine) to 1.6 g of $NaHCO_3$ in 20 mL of water, was added by portions over a period of 30 min. Sodium bromide (660 mg) was added and the solution turned pale orange. Within a few minutes the color of the reaction mixture returned to off-white. Additional sodium hypochlorite (4.7 mL) was added in several portions to drive the reaction to completion. The aqueous layer was separated and extracted with toluene (20 mL) and ethyl acetate (2 x 20 mL). The combined organic extract was washed with a solution of KI (70 mg) in 10% aqueous $KHSO_4$. The organic layer was then washed with 5% $Na_2S_2O_3$ and pH 7 phosphate buffer, dried (Na_2SO_4), and concentrated to give **XIVb** as a pale yellow oil (1.1 g). Spectral data for this compound matched that for the product from Example 6a above.

c. **Aldehyde XIVa (R^2 = Et; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing alcohol **XIVb**, aldehyde **XIVa** was obtained in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.61 (d, J= 3.6 Hz, 1H), 3.48-3.29 (m, 5H), 2.02-1.90 (m, 2H), 1.19 (t, J=7.1 Hz, 3H), 1.14 (t, J= 7.1 Hz, 3H), 0.96 (t, J= 7.4 Hz, 3H).

d. **Aldehyde XIVc (R^2 = *n*-Bu; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing alcohol **XIVb**, aldehyde **XIVc** was obtained in 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.59 (d, J= 3.6 Hz, 1H), 3.48-3.29 (m, 5H), 1.97-1.87 (m, 2H), 1.39-1.22 (m, 4H), 1.18 (t, J=7.2 Hz, 3H), 1.13 (t, J= 7.2 Hz, 3H), 0.90 (t, J= 7.0 Hz, 3H).

e. **Aldehyde XIVd (R^2 = *i*-Bu; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing alcohol **XIVb**, aldehyde **XIVd** was obtained in

96% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.57 (d, *J*= 3.7 Hz, 1H), 3.51-3.27 (m, 5H), 1.83 (t, *J*=7.1 Hz, 3H), 1.66-1.55 (m, 1H), 1.20 (t, *J*= 7.1 Hz, 3H), 1.13 (t, *J*= 7.1 Hz, 3H), 0.93 (d, *J*= 6.6 Hz, 6H).

f. **Aldehyde XIVe (R^2 = CH_2Ph; R^5 = R^6 = Et)**: By a procedure analogous to that described for preparing alcohol **XIVb**, aldehyde **XIVe** was obtained in 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.69 (d, *J*= 2.9 Hz, 1H), 7.29-7.16 (m, 5H), 3.65 (m, 1H), 3.53-3.42 (m, 1H), 3.30 (dd, *J*= 13.5, 9.3 Hz, 1H), 3.23-3.13 (m, 2H), 3.06-2.91 (m, 2H), 1.04 (t, *J*=7.1 Hz, 3H), 0.93 (t, *J*= 7.1 Hz, 3H).

The preparation of clasto-lactacystin β-lactone and analogs thereof according to the synthetic scheme outlined in Scheme 1 as exemplified in Examples 7-9.

Example 7: Aldol adducts (II)

a. **Aldol adduct IIb (R^2 = *n*-Pr; R^1 = *i*-Pr; R^3 = Me; R^4 = Ph; R^5 = R^6 = Et)**: To a cold (-78°C) solution of *trans*-oxazoline **Ia** (R^1 = *i*-Pr; R^4 = Ph) in ether (35 mL) was added lithium bis(trimethylsilyl)amide (2.17 of a 1 M solution in hexane, 2.17 mmol). After 30 min, the orange solution was treated dropwise with a 1M solution of dimethylaluminum chloride in hexane (4.55 mL, 4.55 mmol) and the mixture was stirred for another 60 min before being cooled down to -85°C (liquid N_2 was added to the dry ice/acetone bath). A solution of aldehyde **XIVb** (420 mg, 2.27 mmol) in ether (4 mL) was then added over 10 min along the side of the flask. The mixture was then allowed to warm up to - 40°C over 2.5 h and then quenched by adding 35 mL of saturated aqueous NH_4Cl and 25 mL AcOEt. Enough 2 N HCl was then added until 2 clear phases were obtained (*ca.* 15 mL added). The aqueous layer was extracted with AcOEt (2 x 20 mL) and the combined organic layers were washed successively with 0.5 N aqueous HCl (20 mL), H_2O (20 mL), 0.5 M aqueous $NaHSO_3$ (2 x 15 mL), saturated aqueous $NaHCO_3$ and finally with brine, then dried over Na_2SO_4 and concentrated in vacuo

affording 879 mg (> 100%) of crude Aldol product **IIb** which was pure enough to be used directly in the subsequent step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02-7.97 and 7.53-7.39 (m, 5H), 6.58 (d, *J*= 9.9 Hz, 1H), 4.82 (d, *J*= 2.4 Hz, 1H), 3.73 (s, 3H), 3.69-3.61 (m, 2H), 3.49-3.39 (m, 2H), 3.24-3.16 (m, 1H), 3.05 (m, 1H), 2.89 (m, 1H), 2.28-2.23 (m, 1H), 1.98-1.91 (m, 1H), 1.37-1.20 (m, 6H), 1.19-1.06 (m, 6H), 0.87 (t, *J*= 7.1 Hz, 3H), 0.70 (d, *J*= 6.7 Hz, 3H).

Aldol product **IIb** was also obtained in 100% yield by a procedure analogous to that described above but using cis-oxazoline **Ib** (see below) instead of *trans*-oxazoline **Ia.**

b. Aldol adduct IIb (R^2 = *n*-Pr; R^1 = *i*-Pr; R^3 = Me; R^4 = Ph; R^5 = R^6 = Et): To a cold (-78°C) solution of *trans*-oxazoline **Ia** (R^1 = *i*-Pr; R^4 = Ph) (20.74 g) in THF (280 mL) was added lithium bis(trimethylsilyl)amide (92.4 mL of a 1 M solution in hexane) over 75 min. After 30 min, the orange solution was treated dropwise with a 1M solution of dimethylaluminum chloride in hexane (202 mL) and the mixture was stirred for another 40 min before being cooled down to -85°C (liquid N_2 was added to the dry ice/acetone bath). A solution of aldehyde **XIVb** (19.43 g) in THF (50 mL) was then added over 45 min. The mixture was then allowed to warm to -50°C over 40 min and then to - 20°C over 25 min. The yellow reaction mixture was again cooled to -78°C and then quenched by cautious addition of 40 mL of saturated aqueous NH_4Cl. The reaction mixture was poured slowly into 460 mL of saturated aqueous NH_4Cl. AcOEt (500 mL) was added, and with good stirring the reaction mixture was acidifed with 6 N HCl to produce two clear phases. The aqueous layer was extracted with AcOEt (2 x 200 mL), and the combined organic layers were washed successively with H_2O (2 x 200 mL), saturated aqueous $NaHCO_3$ (2 x 200 mL), and brine (2 x 300 mL). The organic extract was dried over Na_2SO_4 and $MgSO_4$ and concentrated in vacuo to afford 41.55 g of crude Aldol product **IIb** which was pure enough to be used directly in the subsequent step. Spectral data for this compound matched that for the product from Example 7a above.

c. **Aldol adduct IIa (R^2 = Et; R^1 = *i*-Pr; R^3 = Me; R^4 = Ph; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing Aldol adduct **IIb**, the lithium anion of *trans*-oxazoline **Ia** (R^1 = *i*-Pr; R^4 = Ph) was treated successively with dimethylaluminum chloride and aldehyde **XIVa** to provide Aldol adduct **IIa** in 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00-7.97 and 7.51-7.39 (m, 5H), 6.50 (d, *J*= 9.9 Hz, 1H), 4.80 (d, *J*= 2.4 Hz, 1H), 3.81-3.64 (m, 2H), 3.74 (s, 3H), 3.45 (m, 2H), 3.19 (m, 2H), 2.93-2.84 (m, 2H), 2.24 (m, 1H), 1.89 (m, 1H), 1.73-1.64 (m, 4H), 1.29 (t, *J*= 7.2 Hz, 3H), 1.12 (d, *J*= 6.9 Hz, 3H), 1.07 (d, *J*= 7.2 Hz, 3H), 0.70 (d, *J*= 6.7 Hz, 3H).

d. **Aldol adduct IIc (R^2 = *n*-Bu; R^1 = *i*-Pr; R^3 = Me; R^4 = Ph; R^5 = R^6 = Et).** By a procedure analogous to that described for preparing Aldol adduct **IIb**, the lithium anion of *trans*-oxazoline **Ia** (R^1 = *i*-Pr; R^4 = Ph) was treated successively with dimethylaluminum chloride and aldehyde **XIVc** to provide Aldol adduct **IIc** in 100% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02-7.98 and 7.53-7.33 (m, 5H), 6.57 (d, *J*= 10.0 Hz, 1H), 4.81 (d, *J*= 2.3 Hz, 1H), 3.73 (s, 3H), 3.68-3.60 (m, 2H), 3.49-3.17 (m, 2H), 3.00 (m, 1H), 2.90 (m, 1H), 1.98-1.87 (m, 2H), 1.38-0.83 (m, 16H), 0.70 (d, *J*= 6.7 Hz, 3H).

e. **Aldol adduct IId (R^2 = *i*-Bu; R^1 = *i*-Pr; R^3 = Me; R^4 = Ph; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing Aldol adduct **IIb**, the lithium anion of *trans*-oxazoline **Ia** (R^1 = *i*-Pr; R^4 = Ph) was treated successively with dimethylaluminum chloride and aldehyde **XIVd** to provide Aldol adduct **IId** in 100% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01-7.80 and 7.55-7.20 (m, 5H), 4.87 (d, *J*= 2.3 Hz, 1H), 3.73 (s, 3H), 3.69-3.58 (m, 2H), 3.51-3.32 (m, 2H), 2.98-2.87 (m, 1H), 2.33-2.24 (m, 1H), 2.12-2.02 (m, 1H), 1.83 (t, *J*= 7.1 Hz, 1H), 1.35 (t, *J*= 7.1 Hz, 3H), 1.25-1.05 (m, 5H), 0.93 (d, *J*= 6.6 Hz, 3H), 0.89 (d, *J*= 6.5 Hz, 3H), 0.80 (d, *J*= 6.5 Hz, 3H), 0.69 (d, *J*= 6.7 Hz, 3H).

f. **Aldol adduct IIe (R^2 = CH_2Ph; R^1 = *i*-Pr; R^3 = Me; R^4 = Ph; R^5 = R^6 = Et):** By a procedure analogous to that described for preparing Aldol adduct **IIb**, the lithium anion of *trans*-oxazoline **Ia** (R^1 = *i*-Pr; R^4 = Ph) was treated successively with dimethylaluminum chloride and aldehyde **XIVe** to provide Aldol

adduct **IIe** in 100% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01-7.93 and 7.54-7.10 (m, 10H), 4.71 (d, *J*= 2.5 Hz, 1H), 3.73 (s, 3H), 3.68-3.58 (m, 2H), 3.48-2.79 (m, 6H), 2.17 (m, 1H), 1.12-0.91 (m, 9H), 0.68 (d, *J*= 6.7 Hz, 3H).

Example 8: γ-Lactams (IV)

a. γ-Lactam IVb (R^2 = *n*-Pr; R^1 = *i*-Pr; R^3 = Me): A solution of Aldol adduct **IIb** (4.72 g, 10.9 mmol) in 100 mL 1:9 AcOH/MeOH, to which was added 4.8 g 20% $Pd(OH)_2$/C, was vigorously shaken under 55 p.s.i. H_2 for 60 h. The mixture was brought down to atmospheric temperature before being filtered and concentrated *in vacuo*. The solid obtained was purified by flash chromatography (SiO_2, elution with 1% AcOH in 1:1 AcOEt/hexane) affording 2.23 g (75%) of desired γ-lactam **IVb** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (br. s, 1H), 4.77 (br. d, *J*= 11.5 Hz, 1H), 4.47 (dd, *J*= 11.5, 5.6 Hz, 1H), 4.08 (dd, *J*= 9.4, 5.0 Hz, 1H), 3.83 (s, 3H), 2.93 (m, 1H), 1.78-1.39 (m, 6H), 1.02-0.88 (m, 9H).

b. γ-Lactam IVa (R^2 = Et; R^1 = *i*-Pr; R^3 = Me): By a procedure analogous to that described for preparing γ-lactam **IVb**, Aldol adduct **IIa** was hydrogenated at 55 p.s.i. for 48 h to provide γ-lactam **IVa** in 72% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (br. s, 1H), 4.62 (br. d, *J*= 11.2 Hz, 1H), 4.51 (dd, *J*= 11.2, 5.4 Hz, 1H), 3.83 (s, 3H), 2.85 (m, 1H), 1.77-1.64 (m, 3H), 1.01 (t, *J*= 7.4 Hz, 3H), 0.98 (d, *J*= 6.9 Hz, 3H), 0.95 (d, *J*= 6.9 Hz, 3H).

c. γ-Lactam IVc (R^2 = *n*-Bu; R^1 = *i*-Pr; R^3 = Me): A solution of Aldol adduct **IIc** (361 mg, 0.80 mmol) in 6 mL 1:9 AcOH/MeOH, to which was added 250 mg 20% $Pd(OH)_2$/C, was vigorously shaken under 50 p.s.i. H_2 for 24 h. More catalyst (100 mg) was then added and the mixture was again shaken at 50 p.s.i. for another 24 h after which time it brought down to atmospheric temperature before being filtered. The filtrate was then heated to reflux for 30 min, cooled to room temperature and concentrated *in vacuo*. The solid obtained was co-evaporated once with toluene and purified by flash chromatography (SiO_2, elution with 4%

MeOH/$CHCl_3$) affording 140 mg (61%) of desired γ-lactam **IVc** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (br. s, 1H), 4.93 (br. d, J= 11.3 Hz, 1H), 4.46 (dd, J= 11.3, 5.5 Hz, 1H), 4.15-4.08 (m, 1H), 3.83 (s, 3H), 2.94-2.87 (m, 1H), 1.80-1.34 (m, 6H), 0.94 (d, J= 6.9 Hz, 3H), 0.89 (t, J= 7.2 Hz, 3H).

d. γ-Lactam IVd (R^2 = *i*-Bu; R^1 = *i*-Pr; R^3 = Me): By a procedure analogous to that described for preparing γ-lactam **IVc**, Aldol adduct **IId** was hydrogenated at 50 p.s.i. for 40 h and heated to reflux for 30 min providing γ-lactam **IVd** in 61% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (br. s, 1H), 4.81 (br. d, J= 11.5 Hz, 1H), 4.46 (m, 1H), 4.09 (m, 1H), 3.83 (s, 3H), 3.04-2.98 (m, 1H), 1.78-1.73 (m, 2H), 1.66-1.47 (m, 3H), 1.00-0.90 (m, 12H).

e. γ-Lactam IVe (R^2 = CH_2Ph; R^1 = *i*-Pr; R^3 = Me): By a procedure analogous to that described for preparing γ-lactam **IVc**, Aldol adduct **IIe** was hydrogenated at 50 p.s.i. for 24 h and heated to reflux for 30 min providing γ-lactam **IVe** in 71% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (br. s, 1H), 7.35-7.15 (m, 5H), 5.02 (br. d, J= 11.7 Hz, 1H), 4.40-4.34 (m, 1H), 4.06-4.01 (m, 1H), 3.84 (s, 3H), 3.34-3.27 (m, 1H), 3.10-3.04 (m, 2H), 1.84-1.72 (m, 1H), 0.98 (d, J= 6.7 Hz, 3H), 0.93 (d, J= 6.9 Hz, 3H).

Example 9: β-Lactones (VII)

a. β-Lactone VIIb (R^2 = *n*-Pr; R^1 = *i*-Pr): To a cold (0°C) solution of γ-lactam **IVb** (2.20 g, 8.06 mmol) in EtOH (100 mL) was added 0.1N aqueous NaOH (100 mL, 10.0 mmol). The mixture was stirred at room temperature for 15 h after which time H_2O (50 mL) and AcOEt (100 mL) were added. The aqueous layer was then washed with AcOEt (2 x 50 mL), acidified with 6N aqueous HCl and concentrated *in vacuo* to a volume of *ca* 60 mL. This solution was then frozen and lyophilized. The obtained solid was suspended in THF, filtered to get rid of sodium chloride and concentrated *in vacuo* affording 2.05 g (98%) of the desired dihydroxyacid as white solid. ^{1}H NMR (300 MHz, CD_3OD)

δ 4.42 (d, J= 5.8 Hz, 1H), 3.90 (d, J= 6.5 Hz, 1H), 2.84 (m, 1H), 1.70-1.24 (m, 6H), 0.95-0.84 (m, 9H).

To a solution of the dihydroxyacid (1.90 g, 7.33 mmol) in anhydrous THF (36 mL) was added a solution of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 2.59, 8.06 mmol) in anhydrous MeCN (36 mL) followed by triethylamine (0.72 mL, 22.0 mmol). After stirring for 70 min at room temperature, some toluene was added and the mixture was concentrated *in vacuo* and co-evaporated 2 more times with toluene. Purification by flash chromatography (SiO_2, elution with 2:3 AcOEt/hexane) afforded 1.44 g (81%) of desired β-lactone **VIIb** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.07 (br. s, 1H), 5.26 (d, J= 6.1 Hz, 1H), 3.97 (dd, J= 6.4, 4.4 Hz, 1H), 2.70-2.63 (m, 1H), 2.03 (d, J= 6.4 Hz, 3H), 1.93-1.44 (m, 5H), 1.07 (d, J= 7.0 Hz, 3H), 0.99 (d, J= 7.3 Hz, 3H), 0.91 (d, J= 6.7 Hz, 3H). LRMS (FAB) *m/e* 242 ($M+H^+$).

b. **β-Lactone VIIa (R^2 = Et; R^1 = *i*-Pr)**: Hydrolysis of **IVa**, as described for **IVb** above, afforded the corresponding dihydroxyacid in 100% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 4.45 (d, J= 5.8 Hz, 1H), 3.90 (d, J= 6.4 Hz, 1H), 2.74 (m, 1H), 1.71-1.53 (m, 3H), 0.94 (t, J= 7.4 Hz, 3H), 0.92 (d, J= 6.8 Hz, 3H), 0.88 (d, J= 6.8 Hz, 3H).

By a procedure analogous to that described for preparing β-lactone **VIIb**, β-lactone **VIIa** was obtained in 79% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.17 (br. s, 1H), 5.30 (d, J= 6.0 Hz, 1H), 3.98 (dd, J= 6.4, 4.4 Hz, 1H), 2.60 (m, 1H), 2.08 (d, J= 6.4 Hz, 3H), 1.97 (m, 2H), 1.75 (m, 1H), 1.12 (t, J= 7.5 Hz, 3H), 1.07 (d, J= 6.8 Hz, 3H), 0.92 (d, J= 6.8 Hz, 3H).

c. **β-Lactone VIIc (R^2 = *n*-Bu; R^1 = *i*-Pr)**: Hydrolysis of **IVc**, as described for **IVb** above, afforded the corresponding dihydroxyacid in 100% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 4.42 (d, J= 5.8 Hz, 1H), 3.90 (d, J= 6.4 Hz, 1H), 2.86-2.79 (m, 1H), 1.70-1.24 (m, 8H), 0.97-0.86 (m, 9H).

By a procedure analogous to that described for preparing β-lactone **VIIb**, β-lactone **VIIc** was obtained in 40% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.14

(br. s, 1H), 5.27 (d, J= 6.1 Hz, 1H), 3.97 (d, J= 4.4 Hz, 1H), 2.68-2.61 (m, 1H), 1.94-1.86 (m, 2H), 1.72-1.36 (m, 7H), 1.07 (d, J= 7.0 Hz, 3H), 0.93 (t, J= 7.1 Hz, 3H), 0.91 (d, J= 6.8 Hz, 3H). LRMS (FAB) *m/e* 256 ($M+H^+$)

d. **β-Lactone VIId (R^2 = *i*-Bu; R^1 = *i*-Pr)**: Hydrolysis of **IVd**, as described for **IVb** above, afforded the corresponding dihydroxyacid in 100% yield. ^1H NMR (300 MHz, CD_3OD) δ 4.50 (d, J= 5.8 Hz, 1H), 4.00 (d, J= 6.5 Hz, 1H), 3.09-3.02 (m, 1H), 1.90-1.61 (m, 3H), 1.49-1.40 (m, 2H), 1.02 (d, J= 6.7 Hz, 3H), 0.98 (d, J= 6.5 Hz, 3H), 0.97 (d, J= 6.7 Hz, 3H).

By a procedure analogous to that described for preparing β-lactone **VIIb**, β-lactone **VIId** was obtained in 62% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 6.16 (br. s, 1H), 5.25 (d, J= 6.1 Hz, 1H), 3.97 (d, J= 4.4 Hz, 1H), 2.71 (dd, J= 15.1, 6.2 Hz, 1H), 1.95-1.66 (m, 5H), 1.08 (d, J= 6.9 Hz, 3H), 0.99 (d, J= 6.3 Hz, 3H), 0.98 (d, J= 6.3 Hz, 3H), 0.92 (d, J= 6.7 Hz, 3H). LRMS (FAB) *m/e* 256 ($M+H^+$).

e. **β-Lactone VIIe (R^2 = CH_2Ph; R^1 = *i*-Pr)**: Hydrolysis of **IVe**, as described for **IVb** above, afforded the corresponding dihydroxyacid in 88% yield. ^1H NMR (300 MHz, CD_3OD) δ 7.25-7.04 (m, 5H), 4.29 (d, J= 5.7 Hz, 1H), 3.83 (d, J= 6.4 Hz, 1H), 3.01-2.82 (m, 3H), 1.65 (m, 1H), 0.90 (d, J= 6.6 Hz, 3H), 0.86 (d, J= 6.8 Hz, 3H).

By a procedure analogous to that described for preparing β-lactone **VIIb**, β-lactone **VIIe** was obtained in 77% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 7.36-7.20 (m, 5H), 6.57 (br. s, 1H), 5.08 (d, J= 5.4 Hz, 1H), 3.94 (d, J= 4.5 Hz, 1H), 3.25 (d, J= 10.1 Hz, 1H), 3.01-2.89 (m, 2H), 1.92-1.81 (m, 1H), 1.05 (d, J= 6.9 Hz, 3H), 0.86 (d, J= 6.7 Hz, 3H). LRMS (FAB) *m/e* 290 ($M+H^+$).

The preparation of cis-oxazolines and trans-oxazolines according to the synthetic schemes illustrated in Schemes 3 and 4 as illustrated by Examples 10 and 11.

Example 10: cis-Oxazoline (Ia)

a. **Ethyl 3-(isopropyl)propenoate (XV; R^1 = *i*-Pr; R^3 = Me):** To a stirred solution of carbomethoxymethylene triphenylphosphorane (56.04 g, 167.6 mmol) in dry CH_2Cl_2 (168 mL) at 0°C was added dropwise isobutyraldehyde (17.4 mL, 191.6 mmol). After 5 min, the reaction mixture was warmed to room temperature and stirred for 24h. The solvent was removed *in vacuo* and pentane was added to the white oily solid to precipitate triphenylphosphine oxide. The solid was filtered off and the filtrate concentrated *in vacuo*. The procedure was repeated one more time and the crude olefin (20.00 g, 93%) was obtained as a yellow oil that was sufficiently pure for the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (dd, *J*= 15.7, 6.6 Hz, 1H), 5.77 (dd, *J*= 15.7, 1.5 Hz), 3.72 (s, 3H), 2.44 (m, 1H), 1.06 (d, *J*= 6.7 Hz, 6H).

b. **(2*S*, 3*R*)-Methyl 2,3-dihydroxy-3-[isopropyl]propionate (XVIa; R^1 = *i*-Pr; R^3 = Me):** A mixture of AD-mix-β (100.00 g,), methanesulfonamide (6.78 g, 71.3 mmol) and *tert*-butanol-water (1:1, 720 mL) was stirred vigorously at room temperature for 5 min. The reaction mixture was then cooled to 0°C and α,β-unsaturated ester **XV** (R^1 = *i*-Pr; R^3 = Me) (9.14 g, 71.3 mmol) was added dropwise via a Pasteur pipette. After stirring at 0°C for 96 h, Na_2SO_3 (3.0 g) was added, and stirring continued at room temperature for 1 h. The mixture was diluted with ethyl acetate (200 mL) and transferred to a separatory funnel. The organic layer was removed and the aqueous phase extracted with ethyl acetate (2 x 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The yellow oil obtained was passed through a silica gel pad using 1:1 hexane/ethyl acetate affording diol **XVIa** (R^1 = *i*-Pr; R^3 = Me) (11.48 g, 94%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.28 (dd, *J*= 5.6, 1.8 Hz, 1H), 3.80 (s, 3H), 3.48 (m, 1H), 3.28 (m, 1H), 2.33 (d, J= 9.3 Hz, 1H), 1.87 (m, 1H), 1.02 (d, *J*= 6.7 Hz, 3H), 0.95 (d, *J*= 6.7 Hz, 3H).

c. **(2*R*,3*R*)-Methyl 2-bromo-3-dihydroxy-3-(isopropyl)propionate (*XVIIa*; R^1 = *i*-Pr; R^3 = Me):** (2*S*,3*R*)-Methyl 2,3-dihydroxy-3-

[isopropyl]propionate **XVIa** (R^1 = *i*-Pr; R^3 = Me) (1.0 g, 6.17 mmol) and trimethylorthobenzoate (1.02 mL, 80.1 mmol) were dissolved in CH_2Cl_2 (20 mL) and treated with $BF_3 \cdot OEt_2$ (40.0 µL, 0.32 mmol). After stirring for 75 min, the mixture was concentrated under full vacuum (0.05 mm Hg) for 35 min. The mixture was redissolved in CH_2Cl_2 (20.0 mL), cooled to 0°C and treated sequentially with Et_3N (43.0 µL, 0.31 mmol) and acetyl bromide (0.48 mL, 6.49 mmol). After stirring for 4 h at 0°C, the reaction mixture was treated with saturated $NaHCO_3$ solution (12 mL) and allowed to warm up to room temperature. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo* affording the crude α-bromo β-benzoate **XVIIa** (R^1 = *i*-Pr; R^3 = Me) (1.36 g, 85%) as a clear colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05-8.00 (m, 2H), 7.47-7.40 (m, 3H), 5.57 (dd, *J*= 8.8, 3.9 Hz, 1H), 4.47 (d, *J*= 8.8 Hz, 1H), 3.67 (s, 3H), 2.45 (m, 1H), 1.01 (d, J= 6.8 Hz, 6H).

d. (2*S*3*R*)-Methyl 2-azo-3-dihydroxy-3-[isopropyl]propionate (XVIIIa; R^1 = *i*-Pr; R^3 = Me): A solution of (2*R*, 3*R*)-Methyl 2-bromo-3-dihydroxy-3-[isopropyl]propionate **XVIIa** (R^1 = *i*-Pr; R^3 = Me) (2.00 g, 6.07 mmol) in 15 mL DMSO was treated with sodium azide (790.0 mg, 12.2 mmol). After stirring for 12 h at room temperature, the mixture was partitioned between H_2O and ethyl acetate (50 mL each). The aqueous layer was extracted with more ethyl acetate and the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo affording the desired α-azo β-benzoate (1.55 g, 87%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07-8.02 (m, 2H), 7.55-7.43 (m, 3H), 5.40 (dd, *J*= 8.8, 2.8 Hz, 1H), 3.73 (s, 3H), 2.24 (m, 1H), 1.04 (d, *J*= 5.8 Hz, 3H), 0.98 (d, *J*= 5.8 Hz, 3H).

Repeating the same procedure but using DMF as the solvent instead of DMSO afforded the desired α-azo β-benzoate in 85% yield.

e. Benzamide XIXa (R^1 = *i*-Pr; R^3 = Me): A solution of (2*S*, 3*R*)-Methyl 2-azo-3-dihydroxy-3-[isopropyl]propionate **XVIIIa** (R^1 = *i*-Pr; R^3 = Me) (1.50 g, 5.15 mmol) in ethyl acetate (25 mL) was treated with 200 mg of 20% $Pd(OH)_2$/C

and the suspension was stirred vigorously in a H_2 atmosphere under balloon pressure. After 12 hours, the mixture was filtered and refluxed for 4 hours to complete the migration of the benzoyl group. The mixture was then cooled to room temperature and concentrated *in vacuo* affording the desired benzamide (1.25 g, 92%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85-7.83 (m, 2H), 7.46-7.40 (m, 3H), 6.99 (br. d, *J*= 9.1 Hz, 1H), 5.05 (dd, *J*= 9.1, 1.9 Hz, 1H), 3.77 (s, 3H), 1.79 (m, 1H), 1.03 (d, *J*= 6.7 Hz, 3H), 0.99 (d, *J*= 6.7 Hz, 3H).

f. ***cis*-Oxazoline Ia (R^1 = *i*-Pr; R^3 = Me):** A solution of 500 mg of benzamide **XIXa** (R^1 = *i*-Pr; R^3 = Me) (18.8 mmol) in CH_2Cl_2 (20 mL) was treated with 4.50 mL thionyl chloride (61.7 mmol). After stirring at room temperature for 24 h, the mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ solution, dried (Na_2SO_4), concentrated *in vacuo* and chromatographed (silica gel, 1:1 hexane/ethyl acetate) affording the desired *cis*-oxazoline (248 mg, 53%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01-7.97 (m, 2H), 7.52-7.38 (m, 3H), 4.94 (d, *J*= 9.8 Hz, 1H), 4.53 (dd, *J*= 9.8, 7.8 Hz, 1H), 3.76 (s, 3H), 2.09 (m, 1H), 1.05 (d, *J*= 6.5 Hz, 3H), 1.01 (d, *J*= 6.7 Hz, 3H).

Example 11: trans-Oxazoline (Ib)

a. **Ethyl 3-(isopropyl)propenoate (XV; R^1 = *i*-Pr; R^3 = Me):** To a stirred solution of carbomethoxymethylene triphenylphosphorane (56.04 g, 167.6 mmol) in dry CH_2Cl_2 (168 mL) at 0°C was added dropwise isobutyraldehyde (17.4 mL, 191.6 mmol). After 5 min, the reaction mixture was warmed to room temperature and stirred for 24h. The solvent was removed *in vacuo* and pentane was added to the white oily solid to precipitate triphenylphosphine oxide. The solid was filtered off and the filtrate concentrated *in vacuo*. The procedure was repeated one more time and the crude olefin (20.00 g, 93%) was obtained as a yellow oil that was sufficiently pure for the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (dd, *J*= 15.7, 6.6 Hz, 1H), 5.77 (dd, *J*= 15.7, 1.5 Hz), 3.72 (s, 3H), 2.44 (m, 1H), 1.06 (d, *J*= 6.7 Hz, 6H).

b. (2*R*, 3*S*)-Methyl 2,3-dihydroxy-3-[isopropyl]propionate (XVIb; R^1 = *i*-Pr; R^3 = Me): To a clear yellow solution of $K_2OsO_2(OH)_4$ (246.1 mg, 0.67 mmol, 0.95 mol %), hydroquinine 1,4-phthalazinediyl diether (555.1 mg, 0.71 mmol, 1.01 mol %), N-methylmorpholine N-oxide (50 wt % in water, 25.0 mL, 0.106 mol, 1.51 equiv.), *t*-BuOH (84 mL), and H_2O (58 mL) was added at 25°C the neat olefin **XV** (R^1 = *i*-Pr; R^3 = Me) (9.0 g, 70.2 mmol) *via* a syringe pump over a period of 48 h (the syringe was connected to tubing, whose tip was immersed in the solution throughout the reaction time). The resulting clear orange solution was then stirred for another 60 min, after which time ethyl acetate (200 mL) and a solution of Na_2SO_3 (15.0 g) in H_2O (150 mL) were added, and the resulting mixture was stirred for 4 h. The phases were separated, and the aqueous layer was extracted with more ethyl acetate (2x). The organic layers were then combined and the chiral ligand was extracted from the organic phase with a solution of 0.3 M H_2SO_4 in saturated Na_2SO_4 (2 x 100 mL). The phases were once again separated and the aqueous layer was extracted with more ethyl acetate (1x). The organic layers were combined and dried over Na_2SO_4, filtered and concentrated *in vacuo*. This afforded 11.4 g (*ca.* 100%) of a white oily solid which was shown to be 70% e.e (determined by ^{1}H NMR from a 1:1 molar solution of diol and Europium tris[3-(heptafluoropropylhydroxymethylene)-(-)-camphorate] in C_6D_6). Recrystallisation from 35-60°C petroleum ether afforded 6.8 g (60%) of (2*R*,3*S*)-Methyl 2,3-dihydroxy-3-[isopropyl]propionate (**XVIb**; R^1 = *i*-Pr; R^3 = Me) that was *ca.* 100% e.e., obtained as white crystals, mp = 32-34°C; = -110.6° (c 1.04, $CHCl_3$)]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.28 (dd, *J*= 5.6, 1.8 Hz, 1H), 3.80 (s, 3H), 3.48 (m, 1H), 3.28 (m, 1H), 2.33 (d, J= 9.3 Hz, 1H), 1.87 (m, 1H), 1.02 (d, *J*= 6.7 Hz, 3H), 0.95 (d, *J*= 6.7 Hz, 3H).

c. (2*S*,3*S*)-Methyl 2-bromo-3-dihydroxy-3-(isopropyl)propionate (XVIIb; R^1 = *i*-Pr; R^3 = Me): (2*R*, 3*S*)-Methyl 2,3-dihydroxy-3-[isopropyl] propionate **XVIb** (R^1 = *i*-Pr; R^3 = Me) (30.0 g, 185.2 mmol) and trimethylorthobenzoate (41.3 mL, 240.7 mmol) were dissolved in CH_2Cl_2 (400 mL) and treated with BF_3·OEt_2 (1.16 mL, 9.25 mmol). After 2 h, triethylamine

(1.8 mL, 13 mmol) was added, and the mixture was concentrated in vacuo and placed under full vacuum (0.05 mm Hg) for 70 min. The residue was redissolved in CH_2Cl_2 (400 mL), cooled to 0°C and treated dropwise with acetyl bromide (14.3 mL, 194.5 mmol). After 2 h, additional acetyl bromide (0.68 mL, 9.25 mmol) was added. After 30 min, saturated $NaHCO_3$ solution (500 mL) was added and the mixture was stirred vigorously for 5-10 min. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo affording the crude α-bromo β-benzoate **XVIIb** (R^1 = *i*-Pr; R^3 = Me) (66.23 g) as a clear colorless oil, containing ~9.3% by wt. methyl benzoate. For product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05-8.00 (m, 2H), 7.47-7.40 (m, 3H), 5.57 (dd, *J*= 8.8, 3.9 Hz, 1H), 4.47 (d, *J*= 8.8 Hz, 1H), 3.67 (s, 3H), 2.45 (m, 1H), 1.01 (d, J= 6.8 Hz, 6H).

d. (2*R*,3*S*)-Methyl 2-azo-3-dihydroxy-3-[isopropyl]propionate (XVIIIb; R^1 = *i*-Pr; R^3 = Me): Sodium azide (24 g, 370 mmol) was added to 230 mL of DMSO and the mixture was stirred at room temperature overnight. To the resultant solution was added a solution of (2*S*, 3*S*)-Methyl 2-bromo-3-dihydroxy-3-[isopropyl] propionate (**XVIIb**; R^1 = *i*-Pr; R^3 = Me) (61 g, 185 mmol) in 20 mL DMSO. After stirring for 11 h at room temperature, the mixture was poured into water (1.5 L) and ether (200 mL) and stirred vigorously for 10-15 min. Ether (100 mL) was added and the layers were separated. The aqueous layer was extracted with ether (2 x 100 mL) and the combined organic layers were washed with water (2 x 100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated in vacuo affording the crude product (57.5 g), containing approximately 3% starting material and 8% elimination byproduct. For product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07-8.02 (m, 2H), 7.55-7.43 (m, 3H), 5.40 (dd, *J*= 8.8, 2.8 Hz, 1H), 3.73 (s, 3H), 2.24 (m, 1H), 1.04 (d, *J*= 5.8 Hz, 3H), 0.98 (d, *J*= 5.8 Hz, 3H).

e. Benzamide XIXb (R^1 = *i*-Pr; R^3 = Me): To a cold (0-5°C) solution of (2*R*,3*S*)-Methyl 2-azo-3-dihydroxy-3-[isopropyl]propionate **XVIIIb** (R^1 = *i*-Pr;

R^3 = Me) (55 g) in methanol (300 mL) was added 94 mL of 4 M HCl/dioxane and 2.75 g of $Pd(OH)_2/C$. The mixture was purged with hydrogen and stirred at room temperature. The mixture was purged with hydrogen every 30 min to remove the liberated nitrogen. After 4 h, the reaction mixture was purged with nitrogen and additional $Pd(OH)_2/C$ (1.3 g) was added. The reaction mixture was purged with hydrogen and again purged every hour for 4 h. The mixture was filtered and concentrated *in vacuo*. The residue was dissolved in water and extracted with EtOAc. The aqueous layer was basified with Na_2CO_3 and again extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated to give a mixture of *N*- and *O*-benzoylated products, which was used directly in the next step.

f. *trans*-Oxazoline Ib (R^1 = *i*-Pr; R^3 = Me): The crude product **IXb** obtained in Example 10e above (37.3 g, 141 mmol) was dissolved in toluene (350 mL). *p*-Toluenesulfonic acid (2.68 g, 14.1 mmol) was added and the mixture was heated to reflux. Water was removed using a Dean Stark trap. After 3 h, ~2.5 mL of water had been collected. The reaction mixture was cooled, diluted with EtOAc (100 mL), washed successively with saturated $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated. The residue was purified over a pad of silica gel (~400 g), eluting with 25-30% EtOAc-hexanes to provide the *trans*-oxazoline **Ib** (R^1 = *i*-Pr; R^3 = Me). ^1H NMR (300 MHz, $CDCl_3$) δ 8.01-7.97 (m, 2H), 7.52-7.38 (m, 3H), 4.68 (apparent t, *J*= 7 Hz, 1H), 4.57 (d, *J*= 7 Hz, 1H), 3.81 (s, 3H), 2.00-1.93 (m, 1H), 1.04 (d, *J*= 6.7 Hz, 3H), 1.00 (d, *J*= 6.8 Hz, 3H).

Example 12
Inactivation of Proteasome Activity

Purification of 20S proteasome and proteasome activator PA28 was performed as previously described (Dick *et al.*, *J. Biol. Chem. 271*:7273 (1996)).

2 mL of assay buffer (20 mM HEPES, 0.5 mM EDTA, pH 8.0) and Suc-Leu-Leu-Val-Tyr-AMC in dimethyl sulfoxide were added to a 3 mL fluorescent cuvette, and the cuvette was placed in the jacketed cell holder of a Hitachi F-2000 fluorescence spectrophotometer. The temperature was maintained at 37°C by a circulating water bath. 0.34 mg of PA28 were added and the reaction progress was monitored by the increase in fluorescence at 440 nm (λ_{ex} = 380 nm) that accompanies production of free AMC. The progress curves exhibited a lag phase lasting 1-2 min resulting from the slow formation of the 20S-PA28 complex. After reaching a steady state of substrate hydrolysis, lactacystin was added to a final concentration of 1 mM, and the reaction was monitored for 1 h. The fluorescence (F) *versus* time (t) data were collected on a microcomputer using LAB CALC (Galactic) software. k_{inact} values were estimated by a nonlinear least-squares fit of the data to the first order equation:

$$F = A(1 - e^{-kt}) + C$$

where $C = F_{t=0}$ and $A = F_{t=\infty} - F_{t=0}$.

Example 13
Inhibition of Intracellular Protein Degradation in C2C12 Cells

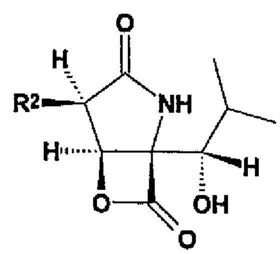
C2C12 cells (a mouse myoblast line) were labeled for 48 hrs with ^{35}S-methionine. The cells were then washed and preincubated for 2 hrs in the same media supplemented with 2 mM unlabelled methionine. The media was removed and replaced with a fresh aliquot of the preincubation media containing 50% serum, and a concentration of the compound to be tested. The media was then removed and made up to 10% TCA and centrifuged. The TCA soluble radioactivity was counted. Inhibition of proteolysis was calculated as the percent decrease in TCA soluble radioactivity. From this data, an IC_{50} for each compound was calculated.

Example 14
Lactone Hydrolysis

The half-lives ($t_{1/2}$) for hydrolysis of β-lactone analogs to the corresponding dihydroxy acids were measured at 37 °C at a concentration of 200 mM in 20 mM HEPES, 0.5 mM EDTA, pH 7.8. Absorbance was measured for at least five half-lives (approximately 1 hour) at 230 nm, the wavelength at which there is the greatest difference in extinction coefficients for the lactone and dihydroxy. Half-lives were calculated using Guggenheim analysis (Gutfreund *Enzymes: Physical Principles*; Wiley and Sons: New York, 1975, pp 118-119). The results of Examples 12-14 are reported in Table 1.

Table 1

Kinetics of Inhibition of 20S Proteasome and Inhibition of Intracellular Protein Degradation



Compound	R^2	Kobs/[I] (M^{-1} s^{-1})[a]	IC_{50} (μM)[b]	$t_{1/2}$ min[c]
2	Me	20,000	0.7-1.1	13
3a	Et	39,000	0.32	15.3
3b	n-Pr	46,500	0.29	15.3
3c	n-Bu	38,000	0.33	17
3d	i-Bu	17,000	0.51	16.8
3e	CH_2Ph	6,400	-	6.8
3f	OMe	82,200	86	3.7

[a]Inactivation of the Chymotrypsin-like activity of PA28-activated 20S proteasome.

[b]Inhibition of intracellular protein degradation in C2C12 cells.

[c]Hydrolysis half-life

The results indicate that the compounds of the present invention are potent inhibitors of the proteasome.

Example 15:
Reduction of Infarct Size and Neuronal Loss

Methods

Male Sprague Dawley rats (250-400 g) were anesthetized with haloethane and subjected to middle cerebral artery (MCA) occlusion using a nylon filament for 2 h. Subsequently, the filament was removed and reperfusion of the infarcted tissue occurred for 24 hours before the rat was sacrificed.

Immediately after the filament was withdrawn, the animals were evaluated using a neurological scoring system. Neurological scores were expressed on a scale from 0 to 10, with 0 representing no neurological deficit and 10 representing severe neurological deficit. After 24 hours and before sacrifice, animals were evaluated a second time using the same neurological scoring system.

Staining of coronal sections (2.0 mm x 7-8) with triphenyltetrazolium chloride (TTC) taken throughout the brain were evaluated under blinded conditions using image analysis to determine infarct size.

Dosing Regimen

Rats were given i.v. bolus injections (1.0 mL/kg) of either vehicle (50% propylene glycol/saline; n=8) or 7-n-propyl-*clasto*-lactacystin β-lactone (**3b**) (0.3 mg/kg; n=7) at 2 hours after the start of the occlusion. Two additional groups of rats were given i.v. bolus injections (1.0 mL/kg) of **3b** at 0 minutes, 2 hours, and 6 hours after the start of the occlusion. One group (0.1 mg/kg x 3; n=6) received 0.1 mg/kg at each of these times, while another group (0.3 mg/kg x 3; n=7) received 0.3 mg/kg at each of the three timepoints.

Results

In animals treated with a single dose of 7-n-propyl-*clasto*-lactacystin β-lactone (**3b**), infarct volume was decreased by 50% (FIG. 1, 0.3 x 1). Infarct volume was not significantly decreased in either the 0.1 mg/kg x 3 dosage group or the 0.3 mg/kg x 3 dosage group (FIG. 1).

All animals had a neurological score of 10 ± 0 immediately after the 2 hour ischemic episode. At 24 hours, the vehicle-treated rats had a mean score of 8.7 ± 0.6, whereas rats treated with a single 0.3 mg/kg dose of 7-n-propyl-*clasto*-lactacystin β-lactone (**3b**) had a mean score of 4 ± 1 (FIG. 2). These data represent a 60% neurological improvement for the drug-treated animals. No significant improvement in neurological score was observed in either the 0.1 mg/kg x 3 dosage group of the 0.3 mg/kg x 3 dosage group (FIG. 2).

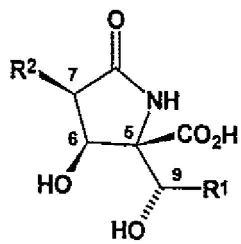
Conclusion

7-n-propyl-*clasto*-lactacystin β-lactone, given once post-ischemia, provides significant protection in both the degree of neurological deficit and infarcted brain damage. From these preliminary data, it appears that a single-dose regimen is preferred over a multiple-dose regimen.

Having now fully described this invention, it will be understood to those of ordinary skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations, and other parameters without affecting the scope of the invention or any embodiment thereof. All patents and publications cited herein are fully incorporated by reference herein in their entirety.

What Is Claimed Is:

1. A process for forming a γ-lactam carboxylic acid of Formula *V*:



V

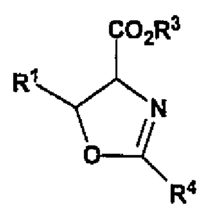
or a salt thereof, wherein

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^2 is alkyl, cycloalkyl, aryl, alkaryl, aralkyl, alkoxy, hydroxy, alkoxyalkyl, or amido, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted; and

said process comprising:

(a) deprotonating a substituted aryl or heteroaryl oxazoline of Formula *I*:



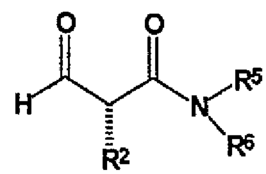
I

where R^1 is as defined above; R^3 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^4 is aryl or heteroaryl, either of which may be optionally substituted;

by treating said substituted aryl or heteroaryl oxazoline with a strong base to form an enolate;

(b) transmetallating said enolate with a metal selected from the group consisting of titanium, aluminum, tin, zinc, magnesium and boron, and thereafter treating with a formyl amide of Formula ***XIV***:

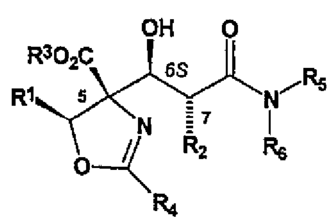


XIV

where R^2 is as defined above, and

R^5 and R^6 are independently one of alkyl or alkaryl; or R^5 and R^6 when taken together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocyclic ring, which may be optionally substituted, and which optionally may include an additional oxygen or nitrogen atom,

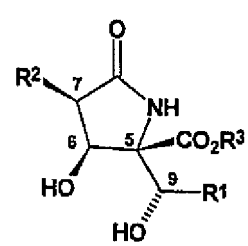
to form an adduct of Formula ***II***:



II;

where R^1 through R^6 are as defined above;

c) catalytically hydrogenating said adduct of Formula ***II*** to form a γ-lactam of Formula ***IV***:

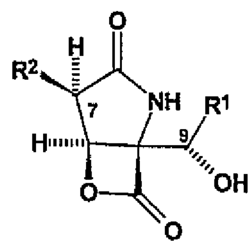


IV

where R^1, R^2 and R^3 are as defined above; and

d) saponifying said γ-lactam of Formula *IV* to form a carboxylic acid of Formula *V*.

2. The process of claim 1, further comprising treating the carboxylic acid of Formula *V* with a cyclizing reagent to form a *clasto*-lactacystin β-lactone of Formula *VII*:

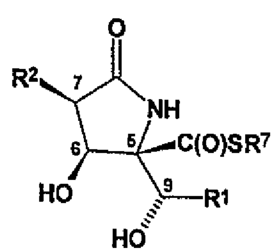


VII

wherein R^1 and R^2 are as defined in claim 1.

3. The process of claim 2, wherein said cyclizing is effected with a reagent selected from the group consisting of aryl sulfonyl chlorides, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate, *O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate and alkyl- aryl- or alkenyl chlorofomates.

4. The process of claim 2, further comprising reacting the *clasto*-lactacystin β-lactone of Formula *VII* with a thiol, R^7SH, to form lactacystin having Formula *VI*:



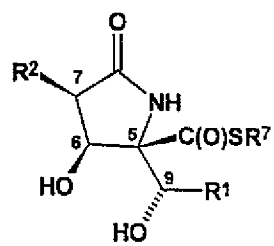
VI

wherein R^1 and R^2 are as defined in claim 1; and

R^7 is alkyl, aryl, aralkyl, alkaryl, wherein any of said alkyl, aryl, aralkyl or alkaryl can be optionally substituted.

5. The process of claim 4, wherein *clasto*-lactacystin β-lactone is converted to lactacystin by treating the β-lactone with *N*-acetylcysteine.

6. The process of claim 1, wherein the carboxylic acid intermediate of Formula *V* is directly coupled to a thiol, R^7SH, to form a lactacystin having Formula *VI*:



VI

wherein R^1 and R^2 are as defined in claim 1; and

R^7 is alkyl, aryl, aralkyl, alkaryl, wherein any of said alkyl, aryl, aralkyl or alkaryl can be optionally substituted.

7. The process of claim 1, wherein in step (a) said strong base is selected from the group consisting of hindered amide bases; alkali metal hexamethyldisilazides; or hindered alkyllithium reagents.

8. The process of claim 1, wherein in step (a) the reaction is conducted at reduced temperature in an ethereal solvent.

9. The process of claim 8, wherein in step (a) said ethereal solvent is selected from the group consisting of diethyl ether, tetrahydrofuran, and

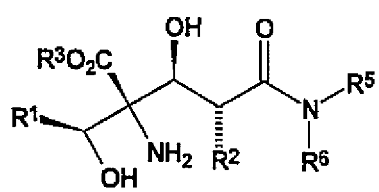
dimethoxyethane, and said reaction temperature is from about -100°C to about -30°C.

10. The process of claim 1, wherein in step (b) said enolate is transmetallated with titanium or aluminum or a mixture thereof.

11. The process of claim 1, wherein in step (b), said enolate is transmetallated by reaction with Me_2AlCl.

12. The process of claim 10, wherein between one and three molar equivalents of said metal are used.

13. The process of claim 1, wherein in step (c) said catalytic hydrogenolysis of the adduct *II*, affords the desired γ-lactam (*IV*) as a mixture with an aminodiol *III*:



III

wherein R^1-R^6 are as defined in claim 1.

14. The process of claim 13, wherein said hydrogenolysis is conducted in the presence of a catalyst selected from the group consisting of palladium black, palladium on activated carbon, and palladium hydroxide on carbon; and in the presence of an organic solvent selected from the group consisting of lower alkanols, lower alkanoates, lower alkanoic acids and mixtures thereof.

15. The process of claim 14, wherein said organic solvent is selected from the group consisting of methanol, ethanol, isopropanol, ethyl acetate, acetic acid, and mixtures thereof.

16. The process of claim 13, wherein the crude product mixture is heated to convert aminodiol ***III*** to the γ-lactam ***IV***.

17. The process of claim 1, wherein

R^1 is C_{1-12} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl;

R^2 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl;

R^3 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl;

R^4 is C_{6-10} aryl, or a heteroaryl group selected from the group consisting of thienyl, benzo[b]thienyl, furyl, pyranyl, isobenzofuranyl, benzoxazolyl, 2*H*-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3*H*-indolyl, indolyl, indazolyl, purinyl, 4*H*-quinolizinyl, isoquinolyl, quinolyl, or triazolyl; and

R^5 and R^6 are independently C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl or together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocycle which can be optionally substituted, and which optionally can include an additional oxygen or nitrogen atom.

18. The process of claim 17, wherein

R^1 is C_{1-6} alkyl, C_{3-6} cycloalkyl, or C_{6-10} aryl;

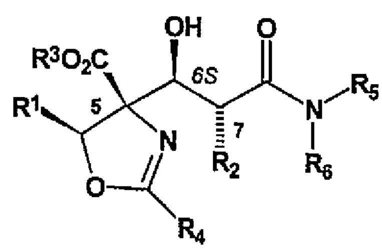
R^2 is methyl, ethyl, propyl, butyl, methoxy, or ethoxy;

R^3 is methyl, ethyl, *tert*-butyl or benzyl;

R^4 is phenyl or phenyl substituted by halogen, C_{1-6} alkyl, C_{1-6} alkoxy, carboxy, or amino; and

NR^5R^6 is one of dimethylamino, diethylamino, pyrrolidino, piperidino, morpholino, or oxazolidinone substituted by halogen, C_{1-6} alkyl, C_{6-10}ar(C_{1-6})alkyl, C_{1-6} alkoxy, carboxy, or amino.

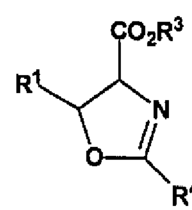
19. A process for forming a substituted oxazoline compound of Formula ***II***:



II;

said method comprising:

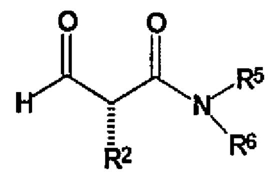
(a) deprotonating a substituted aryl or heteroaryl oxazoline of Formula ***I***:



I

by treating said substituted aryl or heteroaryl oxazoline with a strong base to form an enolate; and

(b) transmetallating said enolate with a metal selected from the group consisting of titanium, aluminum tin, zinc, magnesium and boron, and thereafter reacting with a formyl amide of Formula ***XIV***:



XIV

wherein for each of Formulae ***I***, ***II*** and ***XIV***:

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

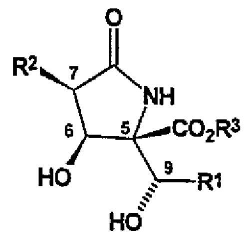
R^2 is alkyl, cycloalkyl, aryl, alkaryl, aralkyl, alkoxy, hydroxy, alkoxyalkyl, or amido, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted;

R^4 is optionally substituted aryl or optionally substituted heteroaryl; and

R^5 and R^6 are independently one of alkyl or alkaryl; or R^5 and R^6 when taken together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocyclic ring, which can be optionally substituted, and which optionally include an additional oxygen or nitrogen atom.

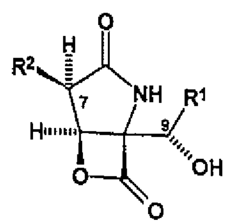
20. The process of claim 19, further comprising catalytically hydrogenating said oxazoline compound of Formula ***II***, and thereafter optionally refluxing the resulting reaction mixture, whereby a β-lactam of Formula ***IV*** is formed:



IV

wherein R^1, R^2 and R^3 are as defined in claim 19.

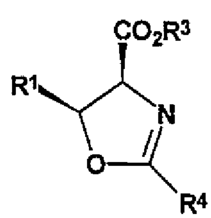
21. The process of claim 20, further comprising saponifying a compound of Formula *IV* and thereafter cyclizing to form a *clasto*-lactacystin β-lactone compound having Formula *VII*:



VII

wherein R^1 and R^2 are as defined in claim 19.

22. A process for forming a substituted aryl oxazoline compound of Formula *Ia*:



Ia

wherein

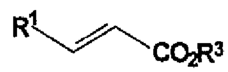
R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^4 is optionally substituted aryl or optionally substituted heteroaryl;

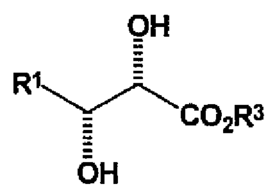
said method comprising:

(a) asymmetrically dihydroxylating an alkene intermediate of Formula ***XV***:



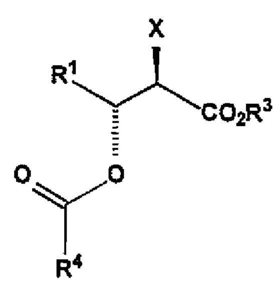
XV

to form an optically active diol of Formula ***XVIa***:



XVIa;

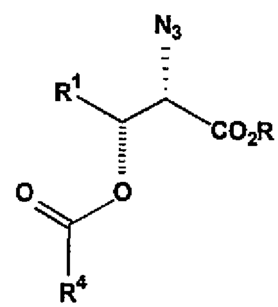
(b) reacting said optically active diol of Formula ***XVIa*** with an orthoester under acid catalysis to give a mixed orthoester, and thereafter reacting the resulting mixed orthoester intermediate with a reagent selected from the group consisting of acyl halides, HCl, HBr, HI, Me_3SiCl, Me_3SiI, Me_3SiBr and halogen-containing Lewis acids to form a haloester derivative of Formula ***XVIIa***:



XVIIa;

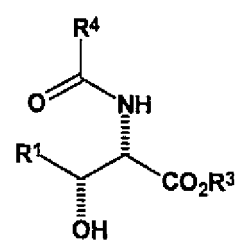
wherein X is Cl, Br, or I;

(c) reacting said haloester derivative with an alkali metal azide to form an azide of Formula ***XVIIIa***:



XVIIIa;

(d) hydrogenating said azide to form a compound of Formula ***XIXa***:



XIXa; and

(e) subjecting the compound of Formula ***XIXa*** to ring closing conditions to form said substituted phenyloxazoline of Formula ***Ia***;
wherein for each of Formulae ***XVa***, ***XVIa***, ***XVIIa***, ***XVIIIa*** and ***XIXa***, R^1, R^3 and R^4 are as defined above for Formula ***Ia***.

23. The process of claim 22, wherein in step (a) the dihydroxylation reaction is conducted with AD-mix-β in the presence of methane sulfonamide to stereoselectively afford the diol of Formula ***XVIa***.

24. The process of claim 22, wherein in step (a) the dihydroxylation reaction is conducted using an N-oxide as a reoxidant.

25. The process of claim 22, wherein in step (b) said diol of Formula ***XVIa*** is treated with an orthoester under Lewis or Brönsted acid catalysis to give a mixed orthoester, which is converted *in situ* to a haloester of Formula ***XVIIa*** wherein X is Br by treatment with acetyl bromide.

26. The process of claim 25, wherein the orthoester employed in this reaction is an aromatic carboxylic acid orthoester.

27. The process of claim 26, wherein the orthoester is trimethyl orthobenzoate.

28. The process of claim 25, wherein said acid catalyst is HBr, $SnCl_4$, $TiCl_4$, BBr_3 or boron trifluoride.

29. The process of claim 22, wherein in step (c) crude haloester of Formula ***XVIIa*** is converted to the azide of Formula ***XVIIIa*** by treatment with an alkali metal azide in a polar aprotic organic solvent.

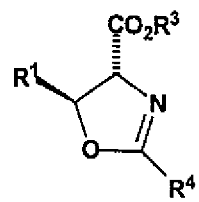
30. The process of claim 22, wherein in step (d) said catalytic hydrogenation of the azide of Formula ***XVIIIa*** is conducted over a palladium catalyst in ethyl acetate.

31. The process of claim 30, wherein said catalytic hydrogenation proceeds with concomitant migration of the aroyl group to afford the hydroxyamide of Formula ***XIXa***.

32. The process of claim 22, wherein in step (e) the hydroxyamide of Formula ***XIXa*** is treated with thionyl chloride in methylene chloride to effect ring closure with inversion of the hydroxyl to produce the *cis*-substituted oxazoline of Formula ***Ia***.

33. The process of claim 32, wherein the *cis*-oxazoline is converted to the *trans*-oxazoline under equilibrating condition by inversion of configuration of the ester substituents.

34. A process for forming a substituted aryl oxazoline compound of Formula ***Ib***:



Ib

wherein

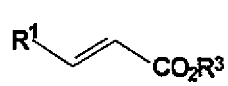
R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^4 is optionally substituted aryl or optionally substituted heteroaryl;

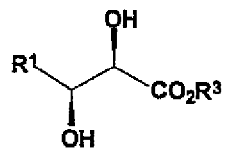
said method comprising:

(a) asymmetrically dihydroxylating an alkene intermediate of Formula ***XV***:



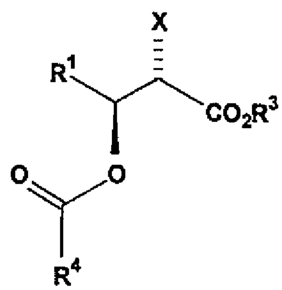
XV

to form an optically active diol of Formula ***XVIb***:



XVIb;

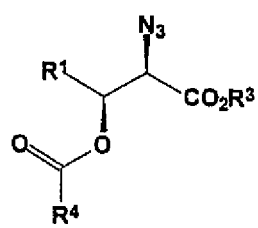
(b) reacting said optically active diol of Formula ***XVIb*** with an orthoester under acid catalysis to give a mixed orthoester, and thereafter reacting the resulting mixed orthoester intermediate with a reagent selected from the group consisting of acyl halides, HCl, HBr, HI,Me_3SiCl, Me_3SiI, Me_3SiBr and halogen-containing Lewis acids to form a haloester derivative of Formula ***XVIIb***:



XVIIb;

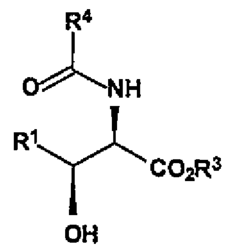
wherein X is Cl, Br, or I;

(c) reacting said haloester derivative with an alkali metal azide to form an azide of Formula ***XVIIIb***:



XVIIIb;

(d) hydrogenating said azide to form a compound of Formula ***XIXb***:



XIXb; and

(e) subjecting the compound of Formula ***XIXb*** to ring closing conditions to form said substituted phenyloxazoline of Formula ***Ib***; wherein for each of Formulae ***XVb***, ***XVIb***, ***XVIIb***, ***XVIIIb*** and ***XIXb***, R^1, R^3 and R^4 are as defined above for Formula ***Ib***.

35. The process of clam 34, wherein in step (a) the dihydroxylation reaction is conducted with AD-mix-α in the presence of methane sulfonamide to stereoselectively afford the diol of Formula ***XVIb***.

36. The process of claim 34, wherein in step (a) the dihydroxylation reaction is conducted using an N-oxide as a reoxidant.

37. The process of claim 34, wherein in step (b) said diol of Formula ***XVIb*** is treated with an orthoester under Lewis or Brönsted acid catalysis to give a mixed orthoester, which is converted *in situ* to a haloester of Formula ***XVIIb*** wherein X is Br by treatment with acetyl bromide.

38. The process of claim 37, wherein the orthoester employed in this reaction is an aromatic carboxylic acid orthoester.

39. The process of claim 38, wherein the orthoester is trimethyl orthobenzoate.

40. The process of claim 37, wherein said acid catalyst is HBr, $SnCl_4$, $TiCl_4$, BBr_3 or boron trifluoride.

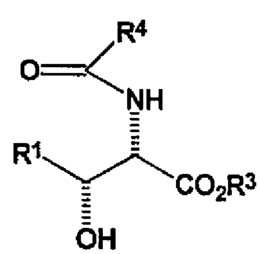
41. The process of claim 34, wherein in step (c) crude haloester of Formula ***XVIIb*** is converted to the azide of Formula ***XVIIIb*** by treatment with an alkali metal azide in a polar aprotic organic solvent.

42. The process of claim 34, wherein in step (d) said catalytic hydrogenation of the azide of Formula ***XVIIIb*** is conducted over a palladium catalyst in ethyl acetate.

43. The process of claim 42, wherein said catalytic hydrogenation proceeds with concomitant migration of the aroyl group to afford the hydroxyamide of Formula ***XIXb***.

44. The process of claim 34, wherein in step (e) the hydroxyamide of Formula ***XIXb*** is treated with thionyl chloride in methylene chloride to effect ring closure with inversion of the hydroxyl to produce the *trans*-substituted oxazoline of Formula ***Ib***.

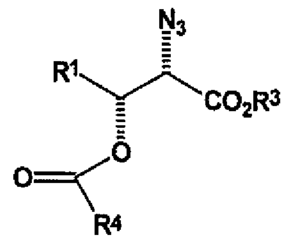
45. A process for forming a compound of Formula ***XIXa***:



XIXa

said method comprising:

hydrogenating an azide compound having Formula ***XVIIIa***:



XVIIIa

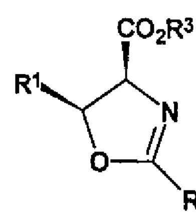
wherein for each of Formulae *XIXa* and *XVIIIa*:

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^4 is aryl or heteroaryl, either of which may be optionally substituted.

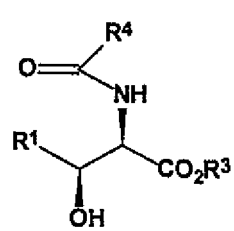
46. The process of claim 45, further comprising subjecting a compound of Formula *XIXa* to ring closing conditions to form a substituted oxazoline compound of Formula *Ia*:



I

wherein R^1, R^3 and R^4 are as defined in claim 45.

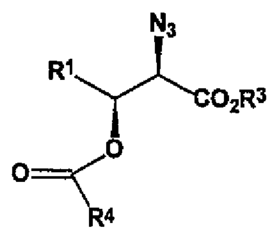
47. A process for forming a compound of Formula *XIXb*:



XIXb

said method comprising:

hydrogenating an azide compound having Formula ***XVIIIb***:



XVIIIb

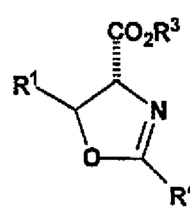
wherein for each of Formulae ***XIXb*** and ***XVIIIb***:

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^4 is aryl or heteroaryl, either of which may be optionally substituted.

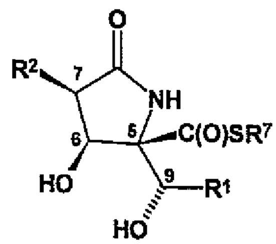
48. The process of claim 47, further comprising subjecting a compound of Formula ***XIXb*** to ring closing conditions to form a substituted oxazoline compound of Formula ***Ib***:



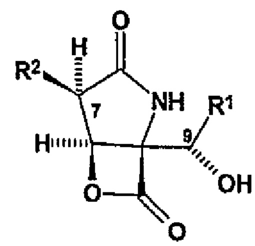
Ib

wherein R^1, R^3 and R^4 are as defined in claim 47.

49. A compound of Formula *VI* or *VII*:



VI



VII

or a salt thereof, wherein:

R^1 is C_{1-12} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl;

R^2 is C_{2-6} alkyl; and

R^7 is alkyl, aryl, aralkyl, alkaryl, wherein any of said alkyl, aryl, aralkyl or alkaryl can be optionally substituted.

50. A compound of claim 49, wherein R^1 is C_{1-4} alkyl.

51. A compound of claim 50, wherein R^1 is isopropyl.

52. A compound of claim 49, wherein R^2 is ethyl, n-propyl, n-butyl or isobutyl.

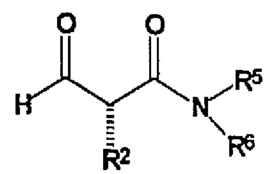
53. A compound of claim 52, wherein R^2 is ethyl.

54. A compound of claim 52, wherein R^2 is n-propyl.

55. A compound of claim 52, wherein R^2 is n-butyl.

56. A compound of claim 52, wherein R^2 is isobutyl.

57. An enantiomerically-enriched formyl amide of Formula ***XIV***:



XIV

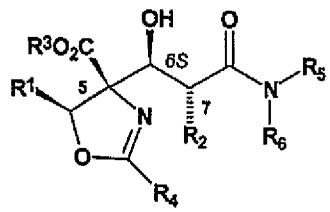
or a salt thereof, wherein

R^2 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl; and

R^5 and R^6 are independently C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, or together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocycle which can be optionally substituted, and which optionally can include an additional oxygen or nitrogen atom.

58. A pharmaceutical composition comprising a compound according to any one of claims 49-56 and a pharmaceutically acceptable carrier or diluent.

59. A compound of Formula ***II***:



II

or a salt thereof, wherein

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^2 is alkyl, cycloalkyl, aryl, alkaryl, aralkyl, alkoxy, hydroxy, alkoxyalkyl, or amido, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted;

R^4 is optionally substituted aryl or optionally substituted heteroaryl; and

R^5 and R^6 are independently one of alkyl or alkaryl; or R^5 and R^6 when taken together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocyclic ring, which can be optionally substituted, and which optionally include an additional oxygen or nitrogen atom.

60. A compound of claim 59, wherein

R^1 is C_{1-12} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

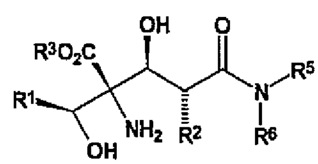
R^2 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, any of which can be optionally substituted;

R^4 is optionally substituted C_{6-10} aryl, or an optionally substituted heteroaryl group selected from the group consisting of thienyl, benzo[β]thienyl, furyl, pyranyl, isobenzofuranyl, benzoxazolyl, 2*H*-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3*H*-indolyl, indolyl, indazolyl, purinyl, 4*H*-quinolizinyl, isoquinolyl, quinolyl, or triazolyl; and

R^5 and R^6 are independently C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl or together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocycle which can be optionally substituted, and which optionally can include an additional oxygen or nitrogen atom.

61. A compound of Formula *III*:



III

or a salt thereof wherein:

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^2 is alkyl, cycloalkyl, aryl, alkaryl, aralkyl, alkoxy, hydroxy, alkoxyalkyl, or amido, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^3 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^5 and R^6 are independently one of alkyl or alkaryl; or R^5 and R^6 when taken together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocyclic ring, which can be optionally substituted, and which can optionally include an additional oxygen or nitrogen atom.

62. A compound of claim 61, wherein

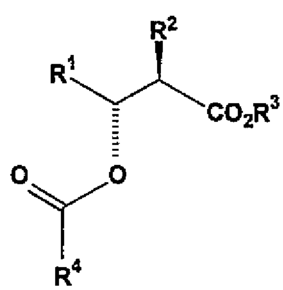
R^1 is C_{1-12} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^2 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10}ar (C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted; and

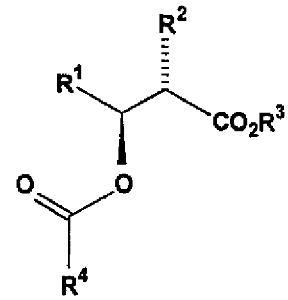
R^3 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{6-14} aryl, C_{6-10}ar (C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, any of which can be optionally substituted; and

R^5 and R^6 are independently C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl, or together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocycle which can be optionally substituted, and which optionally can include an additional oxygen or nitrogen atom.

63. A compound of Formula ***XVIIa*** or ***XVIIb***:



XVIIa



XVIIIb;

or a salt thereof, wherein

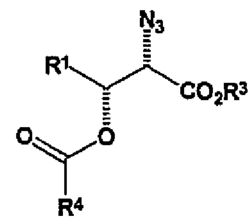
R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^2 is Cl, Br or I;

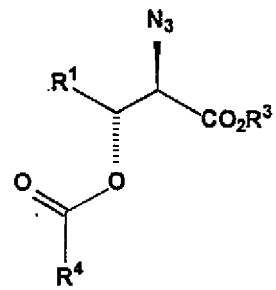
R^3 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^4 is optionally substituted aryl or optionally substituted heteroaryl.

64. A compound of Formula *XVIIIa* or *XVIIIb*



XVIIIa



XVIIIb

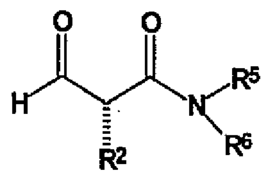
or a salt thereof, wherein

R^1 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, aralkyl, wherein the ring portion of any of said aryl, aralkyl or alkaryl can be optionally substituted;

R^3 is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkaryl, any of which can be optionally substituted; and

R^4 is optionally substituted aryl or optionally substituted heteroaryl.

65. A process for forming an enantiomerically-enriched formyl amide of Formula ***XIV***:



XIV

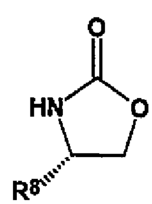
or a salt thereof, wherein

R^2 is alkyl, cycloalkyl, aryl, alkaryl, aralkyl, alkoxy, hydroxy, alkoxyalkyl, or amido, where the ring portion of any of said aryl, aralkyl, or alkaryl can be optionally substituted;

R^5 and R^6 are independently one of alkyl or alkaryl; or R^5 and R^6 when taken together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocyclic ring, which can be optionally substituted, and which optionally include an additional oxygen or nitrogen atom;

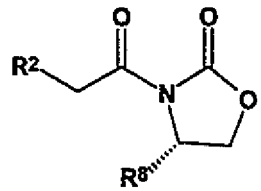
said method comprising:

(a) acylating an anion of a compound of Formula ***VIII:***



VIII

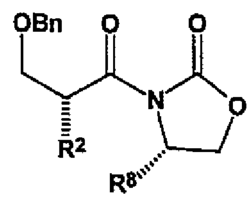
where R^8 is isopropyl or benzyl, with R^2CH_2COCl to form an acyloxazolidinone of Formula ***IX***:



IX

where R^2 and R^8 are as defined above;

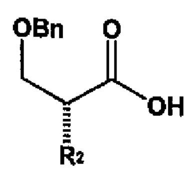
(b) stereoselectively reacting the acyloxazolidinone of Formula *IX* with benzyloxymethyl chloride to form a protected alcohol of Formula *X*:



X

where R^2 and R^8 are as defined above;

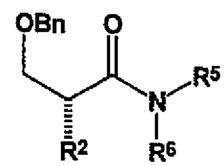
(c) hydrolyzing the protected alcohol of Formula *X* to form a carboxylic acid of Formula *XI*:



XI

where R^2 is as defined above;

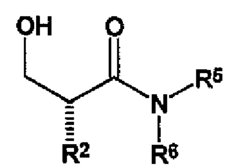
(d) coupling said acid of Formula *XI* with an amine $R^5R^6NH_2$ to provide an amide of Formula *XII*:



XII

where R^2, R^5 and R^6 are as defined above;

(e) catalytically hydrogenating, the amide of Formula *XII* to form an alcohol of Formula *XIII*:



XIII

where R^2, R^5 and R^6 are as defined above; and

(f) oxidizing the resultant alcohol of Formula *XIII* to give a formyl amide of Formula *XIV*.

66. The process of claim 65, wherein:

R^2 is C_{1-8} alkyl, C_{3-8} cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl C_{6-14} aryl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl; and

R^5 and R^6 are independently C_{1-6} alkyl, C_{6-10} ar(C_{1-6})alkyl or C_{1-6}alk(C_{6-10})aryl or together with the nitrogen atom to which they are attached form a 5- to 7-membered heterocycle which can be optionally substituted, and which optionally can include an additional oxygen or nitrogen atom.

67. A method of inhibiting proteasome function in a cell, comprising contacting said cell with a compound of claim 49.

68. A method of inhibiting proteasome function in a mammal, comprising administering to said mammal a compound of claim 49 in an amount effective to inhibit proteasome function.

69. A method of treating inflammation, comprising administering to a subject an effective anti-inflammatory amount of a compound of claim 49.

70. A method of treating cancer, comprising administering to a subject an effective antitumor or antimetastic amount of a compound of claim 49.

71. A method of treating ischemic or reperfusion injury in a mammal comprising administering to said mammal an effective amount of a compound of claim 49.

72. The method of claim 71, wherein the ischemia is the result of vascular occlusion.

73. The method of claim 71, wherein said vascular occlusion occurs during a stroke.

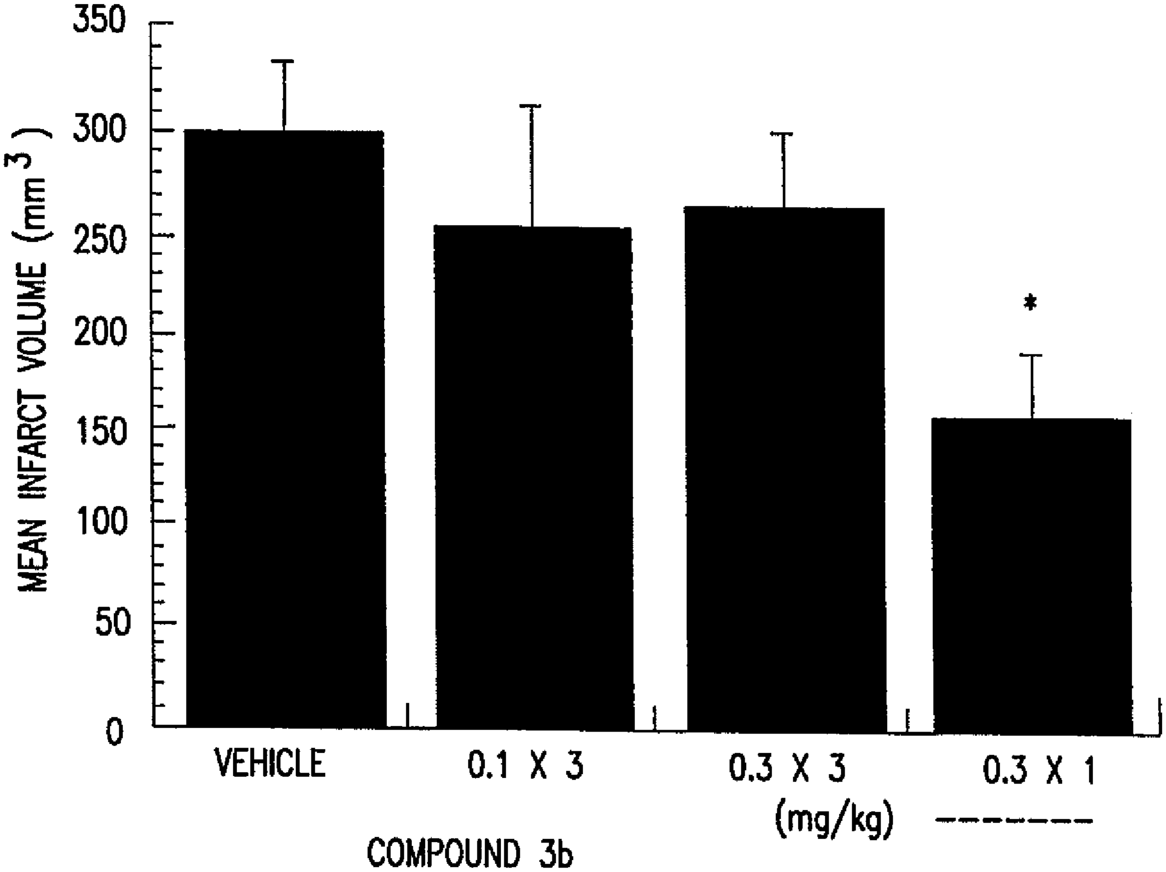

350
300
250
200
150
100
50
0
MEAN INFARCT VOLUME (mm 3)
*
VEHICLE
0.1 X 3
0.3 X 3
0.3 X 1
(mg/kg)
COMPOUND 3b

FIG.1

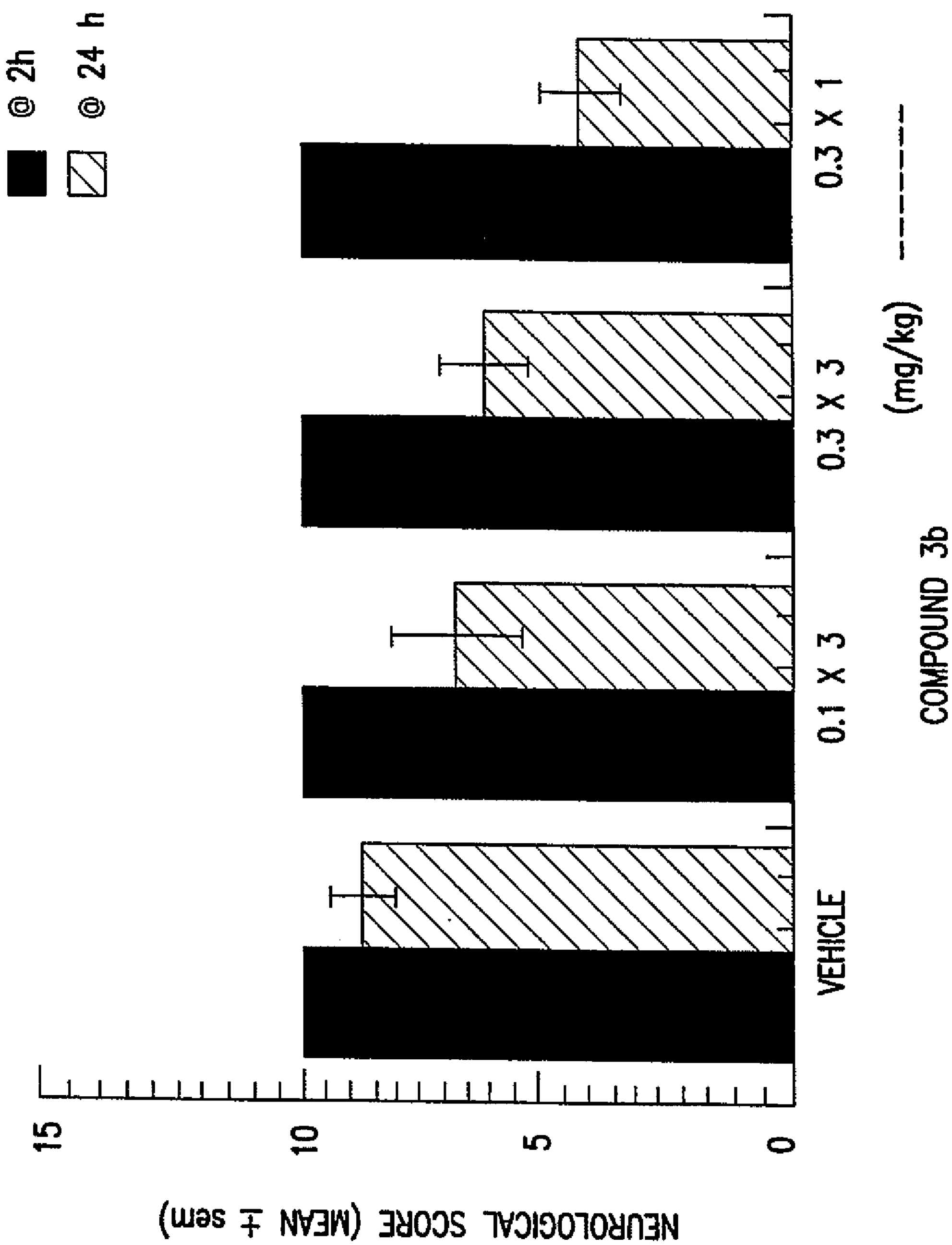

@ 2h
@ 24 h
NEUROLOGICAL SCORE (MEAN ± sem)
15
10
5
0
VEHICLE
0.1 X 3
0.3 X 3
0.3 X 1
(mg/kg)
COMPOUND 3b

FIG. 2

INTERNATIONAL SEARCH REPORT	International application No. PCT/US98/16858

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07D 207/46, 263/14; C07B 21/12; C07C 229/14, 41/06, 221/07
US CL :548/237, 534; 423/365; 560/38, 81; 562/621; 564/502
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 548/237, 534; 423/365; 560/38, 81; 562/621; 564/502

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS
structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	COREY, E.J. et al. Total Synthesis of Lactacystin. J. Am. Chem. Soc. December 1992, Vol. 114, pages 10677-10679, especially page 10678.	1-18, 49-56, 58, 67-73
Y,P -- A	WIPF, P. et al. An Improved Protocol for Azole Synthesis with PEG-supported Burgess Reagent. Tetrahedron Letters. December 1996, Vol 37, No. 27, pages 4659-4662, especially pages 4659.	19-48, 59-60 -------- 1-18, 49-58, 61-73
A,E	U.S. 5,831,068 A (NAIR et al.) 03 November 1998, col. 10, lines 35-68.	1-18, 49-56, 58, 67-73

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 JANUARY 1999	27 JAN 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DOMINIC KEATING Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/16858

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	SHIBATA, K. et al. Kinetic and Thermodynamic Control of L-Threonine Aldolase Catalyzed Reaction and Its Application to the Synthesis of Mycestericin D. Tetrahedron Letters. December 1996, Vol. 37, No. 16, pages 2791-2794, especially pages 2792-2793.	45-48

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-18, 49-56, 58 and 67-73, drawn to compounds of the formula V, their methods of making and methods of use.
Group II, claim(s) 19-44 and 59-60, drawn to compounds of the formula II and their methods of making.
Group III, claim(s) 45-48, drawn to methods of making compounds of the formula XIXa.
Group IV, claim(s)57 and 65-66, drawn to the compounds of the formula XIV and their methods of making.
Group V, claim(s) 61-62, drawn to the compounds of formula III.
Group VI, claim 63, drawn to the compounds of formula XVIIa or XVIIb.
Group VII, claim 64, drawn to the compounds of formula XVIIIa.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical feature of group I is a pyrazole compound, that of group II is an oxazole compound, that of group III is an amide compound, that of group IV is a formyl amide compound, that of group V is an amino compound, that of group VI is an ester compound and that of group VII is an azide compound. Since the special technical features of Groups I-VII are distinct, unity of invention is lacking.